

Polymorphisms in DNA repair and metabolic genes in bladder cancer

Somali Sanyal¹, Fabiola Festa¹, Shigeru Sakano¹,
Zhengzhong Zhang¹, Gunnar Steineck², Ulf Norming³,
Hans Wijkström⁴, Per Larsson², Rajiv Kumar^{1,5,6} and
Kari Hemminki^{1,5}

¹Department of Biosciences at Novum, Karolinska Institute, S-14157 Huddinge, Sweden, ²Clinical Cancer Epidemiology and Clinical Oncology, Department of Oncology-Pathology, Karolinska Hospital, S-171 76 Stockholm, Sweden, ³Department of Urology, Stockholm Southern Hospital, S-118 83 Stockholm, Sweden, ⁴Department of Urology, Huddinge University Hospital, S-141 86 Huddinge, Sweden and ⁵Division of Molecular Genetic Epidemiology German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany

⁶To whom correspondence should be addressed at:
Division of Molecular Genetic Epidemiology, German Cancer Research Center, Im Neuenheimer Feid 580, D-69120 Heidelberg, Germany
Email: rajiv.kumar@cnt.ki.se

We investigated the association of urinary bladder cancer with genetic polymorphisms in the xeroderma pigmentosum complementation group C (*XPC*), group D (*XPB*) and group G (*XPG*), X-ray repair cross-complementing group 1 (*XRCC1*) and group 3 (*XRCC3*), Nijmegen breakage syndrome 1 (*NBS1*), cyclin D1, methylene-tetrahydrofolate reductase (*MTHFR*), NAD(P)H dehydrogenase quinone 1 (*NQO1*), *H-ras* and glutathione S-transferase theta 1 (*GSTT1*) genes. Bladder cancer patients from the different hospitals in Stockholm County Council area and matching controls were genotyped for different polymorphisms. The frequency of the variant allele for A/C polymorphism in exon 15 of the *XPC* gene was significantly higher in the bladder cancer cases than in the controls (OR 1.49, 95% CI 1.16–1.92, $P = 0.001$). The variant allele homozygote genotype for the T/C polymorphism in exon 1 of the *H-ras* gene was associated with a decreased risk for bladder cancer (OR 0.12, 95% CI 0.02–0.67, $P = 0.006$). The variant allele genotypes for the single nucleotide polymorphisms (SNPs) in DNA repair genes, *XPG* and *NBS1*, showed a marginal association with the occurrence of bladder cancer (OR 0.38, 95% CI 0.15–0.94, $P = 0.03$ and OR 1.64, 95% CI 0.92–2.90, $P = 0.09$, respectively). We also report a positive correlation between the null homozygote of *GSTT1* with the risk of bladder cancer (OR 2.54, 95% CI 1.32–4.98, $P = 0.003$). For other polymorphisms included in this study, *NBS1* Glu185Gln, *XPB* Lys751Gln, *XPG* Asp1104His, *XRCC1* Arg399Gln, *XRCC3* Thr241Met, cyclin D1 Pro242Pro, *MTHFR* Ala222Val and Glu429Ala, *NQO1* Arg139Trp

Abbreviations: GSTT1, glutathione S-transferase theta 1; MTHFR, methylene-tetrahydrofolate reductase; NBS1, Nijmegen breakage syndrome 1; NQO1, NAD(P)H dehydrogenase quinone 1; SNP, single nucleotide polymorphism; XPC, xeroderma pigmentosum complementation group C; XPB, xeroderma pigmentosum complementation group D; XPG, xeroderma pigmentosum complementation group G; XRCC1, X-ray repair cross-complementing group 1; XRCC3, X-ray repair cross-complementing group 3.

and Pro187Ser, no significant differences for genotype distributions and allele frequencies between the bladder cancer cases and the controls were observed in the present study.

Introduction

Urinary bladder cancer is the fourth most common neoplasm in men in the western world with urothelial cell carcinoma being the most common sub-type (1). The incidence in Sweden is about 32 for men and nine for women per 10⁵ persons-years (approximately 1500 and 600 cases, respectively) (2). The established risk factors for bladder cancer include cigarette smoking, exposure to industrially related aromatic amines and uptake of drugs like phenacetine, chlornaphrazine and cyclophosphamide (3,4). The exposure to such environmental agents and by-products of cellular metabolism results in damage to DNA, which, if left un-repaired, can lead to the process of carcinogenesis. The entire process leading to DNA damage and subsequent repair of the damage involves a host of enzymes (5). DNA damage itself is a consequence of a balance between activation and detoxification of carcinogens that involves phase I and II metabolic enzymes, many of which are polymorphic (6). The genetic polymorphisms in a number of metabolic enzymes have been found as the modulators of bladder cancer risk (7,8).

The repair of damaged DNA is essential to protect cells against cancer (5). Different pathways of DNA repair operate on specific types of damaged DNA, and each pathway involves numerous molecules (9). Until now, enzymes coded by more than 100 genes have been found in human cells that are implicated in four major DNA repair pathways including nucleotide excision repair (NER), base excision repair (BER), double strand break repair and mismatch repair (10–12). A deficiency in repair capacity due to the defect in genes involved in DNA repair can lead to genomic instability and carcinogenesis. Individuals with a repair capacity below the population mean can be at an increased risk of developing different kinds of cancer. DNA polymorphism can result in a subtle structural alteration of the repair enzymes and modulation of cancer susceptibility (13).

A number of studies have investigated the probable association between modulation of bladder cancer risk and polymorphisms in metabolic, DNA repair and cell-cycle regulation genes (14–20). The genes studied for association between polymorphism and bladder cancer risk include mainly xeroderma pigmentosum complementation group D (*XPB*), X-ray repair cross-complementing group 1 (*XRCC1*) and group 3 (*XRCC3*), cyclin D1 and methylene-tetrahydrofolate reductase (*MTHFR*) with not so clear and in some cases contradictory results. Besides, polymorphisms in the *H-ras* gene have also been associated with the risk of bladder cancer (19,20). Several meta-analyses with pooled data have shown

that individuals with alleles associated with NAT2 slow acetylator phenotypes and individuals with glutathione *S*-transferase M1 null alleles are at an increased risk of bladder cancer (7,8,21). These associations probably reflect the role of gene-environment interactions in the etiology of bladder cancer. Several other studies have also indicated rather ambiguously the role of polymorphisms in other metabolic genes in bladder cancer, including glutathione *S*-transferase theta 1 (*GSTT1*) and NAD(P)H dehydrogenase quinone 1 (*NQO1*) (22,23).

In the present study we set to investigate the relation between genetic polymorphism and associated bladder cancer risk in patients recruited in hospitals from the Stockholm area. We examined the role of polymorphisms in genes involved in DNA repair, metabolism and cell-cycle regulation in a moderate sized single study population. We included polymorphisms with functional relevance in the relevant genes. The selected polymorphisms displayed high enough variant allele frequencies (usually over 20%) allowing us to detect a 2-fold increase or decrease in the relative risk. We studied the association of 12 single nucleotide polymorphisms (SNPs) and one homozygous deletion in 11 different genes with risk of bladder cancer. The SNPs studied included xeroderma pigmentosum complementation group C (*XPC*) exon 15 (Lys939Gln, A/C); *XPD* exon 23 (Lys751Gln, A/C); xeroderma pigmentosum complementation group G (*XPG*) (Asp1104His); *XRCC1* exon 10 (Arg399Gln, G/A); *XRCC3* exon 7 (Thr241Met, T/C); Nijmegen breakage syndrome 1 (*NBS1*) exon 5 (Glu185Gln, G/C); cyclin D1 exon 4

(Pro241Pro, G/A); *MTHFR* exon 4 (Ala222Val, C/T) and exon 7 (Glu429Ala, A/C); *NQO1* exon 4 (Arg139Trp, C/T) and exon 6 (Pro187Ser, C/T) and *H-ras* exon 1 (His27His, T/C). We also examine the effect of *GSTT1* null genotype on the risk of bladder cancer.

Materials and methods

Study subjects

Blood samples from urinary bladder cancer patients were collected in urology clinics of seven hospitals in the Stockholm county council area in years 1995–1996 as part of a collaborative study. A total of 546 patients of Caucasian origin were recruited but blood samples could be collected from only 327 individuals. The mean age of patients in this study was 70 years (range 33–96 years). The controls were chosen from the same geographical area, ethnic background and approximately from the similar age group (24). The DNA from blood samples collected from the cases and the controls was extracted as described (24). Smoking data for the cancer cases and the controls was only partially available, which however, showed roughly an equal distribution (data not shown).

Genotyping by RFLP

Genotyping for polymorphisms in *XPC* Lys939Gln, *XPD* Lys751Gln, *XPG* Asp1104His, *XRCC1* Arg399Gln, *XRCC3* Thr241Met, Cyclin D1 Pro241Pro, *MTHFR* Ala222Val and Glu429Ala, *NQO1* Arg139Trp and Pro187Ser and *H-ras* His27His were detected using PCR-RFLP technique. PCR products were generated by using 10 ng of genomic DNA in 10 µl volume reactions containing 20 mM Tris-HCl, 50 mM KCl, 2.0 mM MgCl₂, 0.11 mM each dNTP, 0.3 µM each primer (Table I) and 0.3 U Platinum *Taq* DNA polymerase (Invitrogen, Paisley, UK). For the amplification of exon 1 of the *H-ras* gene, 5% DMSO was included as a co-solvent. PCR products were digested with the appropriate restriction endonucleases (Fermentas, Vilnius,

Table I. Primers and restriction enzymes used for genotyping various polymorphisms in bladder cancer patients and controls

Gene	Primers sequence	Annealing temp. (°C)	Restriction enzyme
<i>XPC</i> ex 15	F GAT GCA GGA GGT GGA CTC TCT R GTA GTG GGG CAG CAG CAA CT	61	<i>PvuII</i>
<i>XPD</i> ex 23	F CCC CTC TCC CTT TCC TCT GTT R GCT GCC TTC TCC TGC GAT TA	61	<i>PstI</i>
<i>XPG</i> ex 15	F TGG ATT TTT GGG GGA GAC CT R CGG GAG CTT CCT CCT TCA CTG AGT	57	<i>Hsp92II</i>
<i>XRCC1</i> ex10	F GCC CCT CAG ATC ACA CCT AAC R CAT TGC CCA GCA CAG GAT AA	61	<i>MspI</i>
<i>XRCC3</i> ex7	F GCT CGC CTG GTG GTC ATC R CTT CCG CAT CCT GGC TAA AAA	63	<i>Hsp92II</i>
<i>NBS1</i> ex5 ^a	F GAC GTC CAA TTG TAA AGC CAG AAT A R TTC AAT TTG TGG AGG CTG CTT Probe G-allele: AGC AGT TGA GTC CAA Probe C-allele: AGC AGT TCA GTC CAA	60	
Cyclin D1 ex4	F CCG CCT CAC ACG CTT CCT CTC R CCC CAG CCC CAA CCT TGT CAC	63	<i>MspI</i>
<i>MTHFR</i> ex4	F GAG GCT GAC CTG AAG CAC TTG R GTG GGG TGG AGG GAG CTT AT	60	<i>TaqI</i>
<i>MTHFR</i> ex7	F ATT CCT CTT CCC CTG CCT TTG R TCC CAC TTC CAG CAT CAC TC	60	<i>MboII</i>
<i>NQO1</i> ex4	F GAG TCC CTG CCA TTC TGA AAG R GGG AAG CTC CAT CTC AAA CAA	58	<i>MspI</i>
<i>NQO1</i> ex6	F TCT TAC TGA GAA GCC CAG ACC R CAA ATA TTC TCC AGG GGT TTC	53	<i>HinfI</i>
<i>GSTT1</i>	F TCT GCC GCC CGA AAC CTT R ACG TCC TCT TGT CCC CCA TTC	61	
<i>GAPDH</i> ^b	F CAA AGC TTG TGC CCA GAC TGT R CGC CCA ATA CGA CCA AAT CT	61	

^aSNP in the exon 5 of *NBS1* gene was determined by real-time PCR using fluorescent labeled probes.

^b*GAPDH* fragment was amplified in a duplex PCR reaction as an internal control for *GSTT1* null type.

Lithuania) that recognized and cut either wild-type or variant sequences at 37°C for at least 3 h. The digested PCR products were resolved on a 10% polyacrylamide gel (Bio-Rad, Hercules, CA) and stained with ethidium bromide for visualization under UV light. The genotype results were regularly checked and confirmed by direct DNA sequencing of the amplified fragments (25). Most of the assays were carried out including samples with known genotypes as controls. Genotypes for polymorphisms without previously known samples were identified by sequencing twice independently; such samples were subsequently used as controls.

Determination of NBS1 polymorphism by real-time PCR

The polymorphism in *NBS1* exon 5 (Glu185Gln, G/C) was analyzed using the ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA). PCR was carried out in the presence of two fluorescent labeled probes (Table I) in 25 μ l volume reaction with 10 ng genomic DNA, forward and reverse primers and Taqman Universal Master mix (Applied Biosystems). The thermal cycler conditions were set with an initial hold at 95°C for 10 min, followed by 40 cycles consisting of 15 s at 95°C and 1 min at 60°C. One sample of each genotype analyzed by RFLP and confirmed by DNA sequence analysis was used as a reference sample.

GSTT1 genotyping

A duplex PCR assay with a GAPDH fragment as an internal control was used for determination of *GSTT1* null allele homozygotes. The 10 μ l volume reaction included 10 ng genomic DNA, 20 mM Tris-HCl, 50 mM KCl, 2.0 mM MgCl₂, 0.11 mM each dNTP, 0.3 μ M each primer (*GSTT1* and GAPDH, Table I), 5% DMSO and 0.3 U Platinum *Taq* DNA polymerase (Invitrogen) in a total volume of 10 μ l. Products from each PCR reaction were separated on 10% TBE gel (Bio-Rad, Hercules, CA) followed by ethidium bromide staining and visualization under UV light.

Statistical analysis

Differences in genotype distributions and allele frequencies in the bladder cancer cases and the controls were compared for statistical significance using the Chi²-test (Yates corrected). The statistical significance for deviations from Hardy-Weinberg Equilibrium (HWE) were determined using the Pearson Chi²-test. Odds ratios with 95% confidence intervals were calculated for the alleles and genotypes between the bladder cancer cases and the controls. Using our sample size, the study had a power of 90% to detect a 2.5-fold increase of the relative risk assuming a 10% prevalence of the rare allele in the control group. For the variant allele frequency >20%, this study had >90% power to detect a 2.0-fold increase of the relative risk. Bonferroni correction was taken into consideration because of the multiple comparisons carried out in this study. According to this correction *P* value ≤ 0.004 was considered statistically significant.

Results

We have determined the frequency of 12 SNPs and one deletion polymorphism in 11 different genes that included the *XPC*, *XPD*, *XPG*, *XRCC1*, *XRCC3*, *NBS1*, cyclin D1, *MTHFR*, *NQO1*, *H-ras* and *GSTT1* genes in bladder cancer cases and matching controls in order to evaluate the modulation of risk of urinary bladder cancer. The genotype distribution for all the SNPs studied was in accordance with HWE, except for polymorphism in exon 1 of the *H-ras* gene, which showed a significant deviation from HWE in the bladder cancer cases.

The frequency of the variant C-allele, for *XPC* exon 15 was found to be significantly higher in the bladder cancer cases than in the control population (*P* 0.001) and the carriers of this allele had an increased risk of bladder cancer (OR 1.49, 95% CI 1.16–1.92) (Table II). Among the five other DNA repair genes studied, the genotype frequency for homozygote C/C in *XPG* exon 15 showed a significant difference between the cases and the controls (OR 0.38, 95% CI 0.15–0.94, *P* 0.03). Similarly *NBS1* exon 5 showed a marginally significant difference between the bladder cancer cases and the controls (OR 1.64, 95% CI 0.92–2.90, *P* 0.09). However, when the Bonferroni correction for multiple comparisons was taken into consideration, the only statistical significance retained

was for the differences in the allelic frequencies of *XPC* polymorphism between the cases and controls.

Among the metabolic genes, an association was found between the occurrence of urinary bladder cancer and the null allele homozygote of *GSTT1* (OR 2.54, 95% CI, 1.32–4.98, *P* 0.003). The distribution of the variant homozygote for T/C polymorphism in exon 1 of the *H-ras* gene was lower in the cases than in the controls, although the difference was not statistically significant after the correction for multiple comparisons. The variant allele seems to play a protective role against bladder cancer as its frequency in bladder cancer cases (25%) was lower than in controls (30%), although the difference in allele frequency was not statistically significant (Table II).

For other SNPs in DNA repair genes (*XPD* Lys751Gln; *XRCC1* Arg399Gln; *XRCC3* Thr241Met), cyclin D1G870A; *MTHFR* Ala222Val and Glu429Ala, *NQO1* Arg139Trp and Pro187Ser, no statistically significant differences for genotype distributions or for allele frequencies between the patients and the controls were observed in the present study.

Discussion

DNA sequence variants are postulated to be responsible for the observed inter-individual differences in DNA repair and metabolic capacity; therefore, these variants probably modulate individual cancer risk (26,27). Our results from the present case-control study showed that amongst the DNA repair genes, the SNP in the *XPC* gene was strongly associated with an increased risk of bladder cancer. The frequency of the variant C-allele (instead of A-allele) that changes lysine to glutamine at codon 939 was higher in the bladder cancer cases than in the controls; the individuals homozygote for the variant allele were at an almost 2-fold increased risk of bladder cancer. *XPC* is an important component of NER and in complex with hHR23B, it is specifically involved in the recognition of the bases that cannot undergo the Watson-Crick base pairing (28,29). To our knowledge, this is the first case-control study for this exonic polymorphism in *XPC*, although, in an earlier study an intronic insertion deletion poly AT polymorphism (PAT) in the gene has been shown to be associated with increased risk of squamous cell carcinoma of head and neck (30). Interestingly, the variant C-allele at codon 939 has been reported to be in linkage disequilibrium with PAT+ allele in intron 9 and with variant A-allele of another polymorphism at splice site in intron 11 of the *XPC* gene (31). In view of this haplotype linkage, our results assume importance, although it remains to be determined, which of these three variants in the gene modulates the cancer risk. The amino acid change causing SNP in the exon 15 is located in the C-terminus of *XPC*, which plays an important role in excision repair. On the other hand, a homozygous splice site variant in intron 11 of *XPC* causes increased skipping of exon 12. *In vitro* functional studies measuring DNA repair capacities have shown inconclusive and contradictory results (32,33).

No other polymorphisms in any of the DNA repair genes included in the present study showed a statistically significant difference between the bladder cancer cases and the healthy controls. The patients homozygote for variant alleles for Asp1104His *XPG* and Glu185Gln *NBS1* polymorphisms showed statistically significant and marginally significant differences, respectively, when compared with the controls.

Table II. Distribution of genotype and allele frequencies in bladder cancer cases and controls

Gene/SNP	Genotype/allele	Cases (%)	Controls (%)	<i>P</i>	OR ^a	95% CI
XPC	AA	113 (37)	105 (43)		1.00	
Exon 4	AC	141 (46)	117 (48)	0.60	1.12	0.77–1.63
	CC	51 (17)	24 (9)	0.02^b	1.97	1.10–3.57
	A-allele	367 (60)	372 (66)			
	C-allele	243 (40)	165 (34)	0.001^a	1.49	1.16–1.92
XPD	AA	110 (36)	95 (39)		1.00	
Exon 23	AC	141 (46)	114 (46)	0.79	1.07	0.73–1.57
	CC	56 (18)	37 (15)	0.35	1.31	0.77–2.22
	A-allele	361 (59)	304 (62)			
	C-allele	253 (41)	188 (38)	0.34	1.13	0.88–1.46
XPG	GG	182 (61)	173 (61)		1.00	
Exon 15	GC	109 (36)	91 (32)	0.51	1.14	0.79–1.64
	CC	8 (3)	20 (7)	0.03^b	0.38	0.15–0.94
	G-allele	473 (79)	437 (77)			
	C-allele	125 (21)	131 (23)	0.41	0.88	0.66–1.17
XRCC1	GG	124 (40)	113 (46)		1.00	
Exon 10	GA	155 (50)	110 (45)	0.19	1.28	0.89–1.86
	AA	32 (10)	23 (9)	0.52	1.27	0.67–2.39
	G-allele	403 (65)	336 (68)			
	A-allele	219 (35)	156 (32)	0.24	1.17	0.90–1.52
XRCC3	CC	131 (43)	107 (44)		1.00	
Exon 7	CT	129 (40)	109 (44)	0.92	0.97	0.66–1.41
	TT	51 (17)	30 (12)	0.26	1.31	0.80–2.41
	C-allele	391 (63)	323 (66)			
	T-allele	231 (37)	169 (34)	0.36	1.13	0.88–1.46
NBS1	GG	114 (38)	116 (42)		1.00	
Exon 5	GC	140 (47)	134 (48)	0.97	1.02	0.72–1.45
	CC	45 (15)	28 (10)	0.09	1.64	0.92–2.90
	G-allele	368 (62)	366 (66)			
	C-allele	230 (38)	190 (34)	0.15	1.20	0.94–1.54
Cyclin D1	GG	87 (28)	61 (25)		1.00	
Exon 4	GA	146 (48)	125 (51)	0.38	0.91	0.53–1.25
	AA	74 (24)	60 (24)	0.62	0.86	0.52–1.43
	G-allele	320 (52)	247 (50)			
	A-allele	294 (48)	245 (50)	0.56	0.93	0.73–1.18
MTHFR	CC	173 (56)	121 (49)		1.00	
Exon 4	CT	113 (37)	102 (42)	0.18	0.77	0.54–1.12
	TT	23 (7)	23 (9)	0.33	0.70	0.36–1.36
	C-allele	459 (74)	344 (70)			
	T-allele	159 (26)	148 (30)	0.12	0.81	0.61–1.06
MTHFR	AA	145 (47)	110 (45)		1.00	
Exon 7	AC	133 (43)	111 (45)	0.66	0.91	0.63–1.31
	CC	33 (11)	24 (10)	0.99	1.04	0.56–1.94
	A-allele	423 (68)	331 (68)			
	C-allele	199 (32)	159 (32)	0.92	0.98	0.75–1.27
NQO1	CC	276 (93)	112 (91)		1.00	
Exon 4	CT	25 (8)	11 (9)	0.98	0.92	0.42–2.06
	TT	0	0	–	–	–
	C-allele	577 (96)	235 (96)			
	T-allele	25 (4)	11 (4)	0.98	0.93	0.42–2.07
NQO1	CC	206 (69)	83 (67)		1.00	
Exon 6	CT	85 (28)	34 (27)	0.92	1.01	0.61–1.66
	TT	8 (3)	7 (6)	0.15	0.46	0.15–1.46
	C-allele	497 (83)	200 (81)			
	T-allele	101 (17)	48 (19)	0.44	0.85	0.57–1.26
GSTT1	Positive	204 (76)	110 (88)			
	Negative	66 (24)	12 (12)	0.003^a	2.54	1.32–4.98
H-ras	TT	153 (51)	54 (45)		1.00	
Exon 1	TC	147 (49)	61 (50)	0.39	0.85	0.54–1.34
	CC	2 (1)	6 (5)	0.006^b	0.12	0.02–0.67
	T-allele	453 (75)	169 (70)			
	C-allele	151 (25)	73 (30)	0.14	0.77	0.55–1.09

Statistically significant differences between cases and controls are shown in bold.

^aDifferences statistically significant after Bonferroni correction as *P* values are <0.004.

^bDifferences not significant after application of Bonferroni correction as *P* values are >0.004.

Individuals with homozygote genotype for SNP at exon 15 of the *XPG* gene showed a decreased risk of bladder cancer. However, these differences after the Bonferroni correction for multiple comparisons did not retain the statistical significance. In an earlier study we reported a marginal association of the variant allele in the *XPG* gene with a risk of breast cancer (25). Similar to earlier studies, we found no evidence of an association between polymorphisms in the *XPD*, *XRCC1* and *XRCC3* genes and bladder cancer risk (14–16).

Polymorphisms in metabolic enzymes that are involved with *in vivo* detoxification of carcinogens have been associated with risk of various cancers (6). In particular, GST M1 and T1, involved in the detoxification of polycyclic aromatic hydrocarbon, and *N*-acetyl transferase-2, involved in acetylation of arylamines, are of interest in bladder cancer. Meta-analyses have shown that GSTM1 deficiency and slow acetylation are major determinants of bladder cancer susceptibility (7,8). Our results show that the deficiency of the GSTT1 also confers an increased risk of bladder cancer. The polymorphisms in the *NQO1* gene did not show any association in this study.

Interestingly, our results show that homozygote carriers of the variant C-allele at the site of C/T polymorphism at codon 27 of the *H-ras* gene have a decreased risk of bladder cancer, but not statistically significant after Bonferroni correction. An earlier report found an opposite effect of this polymorphism on bladder cancer risk, taken together with the fact that this is a silent polymorphism; it is doubtful whether this SNP has any effect on the risk of bladder cancer (19). Other non-coding variants of *H-ras* have been associated with the risk of many cancers; the rare alleles of *H-ras* variable tandem repeats have been also associated with bladder cancer risk (20). The variable tandem repeat located downstream of exon 4 of the *H-ras* gene can possibly have an effect on the transcriptional activity (34). The polymorphism in the other two genes included in the study, *cyclin D1* and *MTHFR*, did not show any difference in the frequency between the cases and the controls. The polymorphism at the splice site in exon 4 of the *cyclin D1* gene, which results in increased alternate splicing, has been associated with an increased risk of bladder cancer in one study while another study did not show such an association (17,18,35).

In conclusion, the results of this study based on the study of 13 polymorphisms in 11 genes show that the variant allele in exon 15 of the *XPC* gene is associated with an increased risk of bladder cancer, although the functional explanation for such an association remains undetermined. In addition, we report an association of increased bladder cancer risk with the *GSTT1* null genotype.

Acknowledgement

This study was partially supported by an EU grant ASHRAM (QLK4-CT-2001-00264).

References

- Knowles, M.A. (1999) The genetics of transitional cell carcinoma: progress and potential clinical application. *BJU Int.*, **84**, 412–427.
- Berggren, P., Steineck, G., Adolfsson, J., Hansson, J., Jansson, O., Larsson, P., Sandstedt, B., Wijkstrom, H. and Hemminki, K. (2001) p53 mutations in urinary bladder cancer. *Br. J. Cancer*, **84**, 1505–1511.
- Cohen, S.M., Shirai, T. and Steineck, G. (2000) Epidemiology and etiology of premalignant and malignant urothelial changes. *Scand. J. Urol. Nephrol. Suppl.*, 105–115.
- Steineck, G., Wiholm, B.E. and Gerhardsson de Verdier, M. (1995) Acetaminophen, some other drugs, some diseases and the risk of transitional cell carcinoma. A population-based case-control study. *Acta Oncol.*, **34**, 741–748.
- Friedberg, E.C. (2003) DNA damage and repair. *Nature*, **421**, 436–440.
- Brockmoller, J., Cascorbi, I., Henning, S., Meisel, C. and Roots, I. (2000) Molecular genetics of cancer susceptibility. *Pharmacology*, **61**, 212–227.
- Johns, L.E. and Houlston, R.S. (2000) *N*-Acetyl transferase-2 and bladder cancer risk: a meta-analysis. *Environ. Mol. Mutagen.*, **36**, 221–227.
- Johns, L.E. and Houlston, R.S. (2000) Glutathione *S*-transferase mu1 (GSTM1) status and bladder cancer risk: a meta-analysis. *Mutagenesis*, **15**, 399–404.
- Goode, E.L., Ulrich, C.M. and Potter, J.D. (2002) Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 1513–1530.
- Friedberg, E.C. (2001) How nucleotide excision repair protects against cancer. *Nature Rev. Cancer*, **1**, 22–33.
- Tauchi, H., Kobayashi, J., Morishima, K. et al. (2002) Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells. *Nature*, **420**, 93–98.
- Wood, R.D., Mitchell, M., Sgouros, J. and Lindahl, T. (2001) Human DNA repair genes. *Science*, **291**, 1284–1289.
- Benhamou, S. and Sarasin, A. (2000) Variability in nucleotide excision repair and cancer risk: a review. *Mutat. Res.*, **462**, 149–158.
- Stern, M.C., Johnson, L.R., Bell, D.A. and Taylor, J.A. (2002) XPD codon 751 polymorphism, metabolism genes, smoking and bladder cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 1004–1011.
- Stern, M.C., Umbach, D.M., van Gils, C.H., Lunn, R.M. and Taylor, J.A. (2001) DNA repair gene XRCC1 polymorphisms, smoking and bladder cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 125–131.
- Stern, M.C., Umbach, D.M., Lunn, R.M. and Taylor, J.A. (2002) DNA repair gene XRCC3 codon 241 polymorphism, its interaction with smoking and XRCC1 polymorphisms and bladder cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 939–943.
- Cortessis, V.K., Siegmund, K., Xue, S., Ross, R.K. and Yu, M.C. (2003) A case-control study of cyclin D1 CCND1 870A → G polymorphism and bladder cancer. *Carcinogenesis*, **24**, 1645–1650.
- Wang, L., Habuchi, T., Takahashi, T. et al. (2002) Cyclin D1 gene polymorphism is associated with an increased risk of urinary bladder cancer. *Carcinogenesis*, **23**, 257–264.
- Johne, A., Roots, I. and Brockmoller, J. (2003) A single nucleotide polymorphism in the human *H-ras* proto-oncogene determines the risk of urinary bladder cancer. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 68–70.
- van Gils, C.H., Conway, K., Li, Y. and Taylor, J.A. (2002) HRAS1 variable number of tandem repeats polymorphism and risk of bladder cancer. *Int. J. Cancer*, **100**, 414–418.
- Engel, L.S., Taioli, E., Pfeiffer, R. et al. (2002) Pooled analysis and meta-analysis of glutathione *S*-transferase M1 and bladder cancer: a HuGE review. *Am. J. Epidemiol.*, **156**, 95–109.
- Park, S.J., Zhao, H., Spitz, M.R., Grossman, H.B. and Wu, X. (2003) An association between NQO1 genetic polymorphism and risk of bladder cancer. *Mutat. Res.*, **536**, 131–137.
- Lee, S.J., Cho, S.H., Park, S.K. et al. (2002) Combined effect of glutathione *S*-transferase M1 and T1 genotypes on bladder cancer risk. *Cancer Lett.*, **177**, 173–179.
- Sakano, S., Berggren, P., Kumar, R., Steineck, G., Adolfsson, J., Onelov, E., Hemminki, K. and Larsson, P. (2003) Clinical course of bladder neoplasms and single nucleotide polymorphisms in the CDKN2A gene. *Int. J. Cancer*, **104**, 98–103.
- Kumar, R., Hoglund, L., Zhao, C., Forsti, A., Snellman, E. and Hemminki, K. (2003) Single nucleotide polymorphisms in the XPG gene: determination of role in DNA repair and breast cancer risk. *Int. J. Cancer*, **103**, 671–675.
- Berwick, M. and Vineis, P. (2000) Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J. Natl Cancer Inst.*, **92**, 874–897.
- Mohrenweiser, H.W. and Jones, I.M. (1998) Variation in DNA repair is a factor in cancer susceptibility: a paradigm for the promises and perils of individual and population risk estimation? *Mutat. Res.*, **400**, 15–24.
- Sugasawa, K., Shimizu, Y., Iwai, S. and Hanaoka, F. (2002) A molecular mechanism for DNA damage recognition by the xeroderma pigmentosum group C protein complex. *DNA Rep.*, **1**, 95–107.

29. Janicijevic, A., Sugawara, K., Shimizu, Y., Hanaoka, F., Wijgers, N., Djurica, M., Hoeijmakers, J.H. and Wyman, C. (2003) DNA bending by the human damage recognition complex XPC-HR23B. *DNA Rep.*, **2**, 325–336.
30. Shen, H., Sturgis, E.M., Khan, S.G. *et al.* (2001) An intronic poly (AT) polymorphism of the DNA repair gene XPC and risk of squamous cell carcinoma of the head and neck: a case-control study. *Cancer Res.*, **61**, 3321–3325.
31. Khan, S.G., Muniz-Medina, V., Shahlavi, T., Baker, C.C., Inui, H., Ueda, T., Emmert, S., Schneider, T.D. and Kraemer, K.H. (2002) The human XPC DNA repair gene: arrangement, splice site information content and influence of a single nucleotide polymorphism in a splice acceptor site on alternative splicing and function. *Nucleic Acids Res.*, **30**, 3624–3631.
32. Qiao, Y., Spitz, M.R., Shen, H., Guo, Z., Shete, S., Hedayati, M., Grossman, L., Mohrenweiser, H. and Wei, Q. (2002) Modulation of repair of ultraviolet damage in the host-cell reactivation assay by polymorphic XPC and XPD/ERCC2 genotypes. *Carcinogenesis*, **23**, 295–299.
33. Khan, S.G., Metter, E.J., Tarone, R.E., Bohr, V.A., Grossman, L., Hedayati, M., Bale, S.J., Emmert, S. and Kraemer, K.H. (2000) A new xeroderma pigmentosum group C poly (AT) insertion/deletion polymorphism. *Carcinogenesis*, **21**, 1821–1825.
34. Green, M. and Krontiris, T.G. (1993) Allelic variation of reporter gene activation by the HRAS1 minisatellite. *Genomics*, **17**, 429–434.
35. Sawa, H., Ohshima, T.A., Ukita, H., Murakami, H., Chiba, Y., Kamada, H., Hara, M. and Saito, I. (1998) Alternatively spliced forms of cyclin D1 modulate entry into the cell cycle in an inverse manner. *Oncogene*, **16**, 1701–1712.

Received September 3, 2003; revised November 26, 2003;
accepted December 9, 2003