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ORIGINAL INVESTIGATION

Genetic variation in the base excision repair pathway and bladder cancer risk

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Abstract Genetic polymorphisms in DNA repair genes may impact individual variation in DNA repair capacity and alter cancer risk. In order to examine the association of common genetic variation in the base-excision repair (BER) pathway with bladder cancer risk, we analyzed 43 single nucleotide polymorphisms (SNPs) in 12 BER genes (*OGG1, MUTYH, APEX1, PARP1, PARP3, PARP4, XRCC1, POLB, POLD1, PCNA, LIG1*, and *LIG3*). Using genotype data from 1,150 cases of urinary bladder transitional cell carcinomas and 1,149 controls from the Spanish Bladder Cancer Study we estimated odds ratios (ORs) and 95% confidence intervals (CIs) adjusting for age, gender, region and smoking status. SNPs in three genes showed significant associations with bladder cancer risk: the 8-oxoG DNA glycosylase gene (*OGG1)*, the Poly (ADP-ribose) polymerase family member 1 (*PARP1*) and the major gap filling polymerase-β (*POLB)*. Subjects who were heterozygous or homozygous variant for an *OGG1* SNP in the promoter region (rs125701) had significantly decreased bladder cancer risk compared to common homozygous: OR (95%CI) 0.78 (0.63–0.96). Heterozygous or homozygous individuals for the functional SNP *PARP1* rs1136410 (V762A) or for the intronic SNP *POLB* rs3136717 were at increased risk compared to those homozygous for the common alleles: 1.24 (1.02–1.51) and 1.30 (1.04–1.62), respectively. In summary, data from this large case-control study suggested bladder cancer risk associations with selected BER SNPs, which need to be confirmed in other study populations.

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Introduction

DNA is damaged from exposure to exogenous and endogenous mutagens, and genetic variation in DNA

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repair genes may alter repair function and contribute to cancer risk (García-Closas et al. 2006a; Goode et al. 2002). Among the various DNA repair pathways, base excision repair (BER) is considered to play a key role by removing DNA damage from oxidation, deamination, and ring fragmentation (Frosina 2004). Exposure to tobacco smoking can increase production of reactive oxygen species (ROS), which have the potential to induce oxidative damage (Wilson et al. 2003), and variation in BER ability may be associated with bladder cancer risk.

BER is primarily responsible for repairing single nucleotides and principally consists of four steps: (1) excising the damaged base by glycosylases (*OGG1* and *MUTYH*); (2) incising the DNA backbone by an endonuclease (*APEX1* also known as *APE*); (3) filling the nucleotide gap by polymerases (*POLB* and *POLD*) coordinated by the *XRCC1* scaffold protein, the protein-modifying poly ADP ribose proteins (e.g. *PARP1*), and the replication component *PCNA* for long base repair patches; and (4) ligating the remaining nick by ligases (*LIG1* and *LIG3*) (Fan and Wilson 2005; Frosina 2004).

Polymorphisms in BER genes that could potentially alter function have been evaluated in a number of epidemiological studies in relation to bladder cancer risk (Andrew et al. 2005; Kelsey et al. 2004; Matullo et al. 2001; Sanyal et al. 2004; Shen et al. 2003; Stern et al. 2001; Wu et al. 2006). The most studied polymorphism is *XRCC1* rs25487 (Q399R), suggesting a decreased risk for individuals with the variant homozygote genotype (Kelsey et al. 2004; Matullo et al. 2001; Sanyal et al. 2004; Shen et al. 2003; Stern et al. 2001; Wu et al. 2006). Although studies have suggested that BER polymorphisms may be related to bladder cancer risk, current evidence is inconclusive and further research is required. In order to determine if genetic variation in BER genes is associated with bladder cancer risk, we evaluated 43 SNPs in 12 genes (*OGG1, MUTYH, APEX1, PARP1, PARP3, PARP4, XRCC1, LIG1, LIG3, POLB, POLD1, PCNA*) in the Spanish Bladder Cancer Study. In addition, we performed a meta-analysis on *XRCC1* rs25487 (Q399R) to clarify the reduced risk in bladder cancer among individuals carrying the variant homozygote genotype suggested by previous reports.

Methods

Study population

The design of the Spanish Bladder Cancer Study has been previously described (García-Closas et al. 2005). Briefly, cases were patients diagnosed with histologically confirmed transitional carcinoma of the urinary bladder in 1998–2001, aged 21–80 years (mean (SD) = 66 (10) years), of which 87% were males. Controls were selected from patients admitted to participating hospitals for diagnoses thought to be unrelated to the exposures of interest, and individually matched to the cases on age at interview within 5-year categories, gender, ethnicity and region. Demographic and risk factor information was collected at the hospitals using computer-assisted personal interviews (CAPI).

A total of 1,219 cases and 1,271 controls, representing 84% of eligible cases and 88% of eligible controls were interviewed. Of these subjects, 1,188 (97%) cases and 1,173 (92%) controls provided a blood or buccal cell sample for DNA extraction. The final study population available for analysis included 1,150 cases and 1,149 controls once subjects were excluded for low DNA yield or quality, missing smoking information, non-transitional urothelial tumors, and non-Caucasian descent. Informed consent was obtained from study participants in accordance with the National Cancer Institute and local Institutional Review Boards.

Subjects were categorized as never smokers (29% of controls) if they smoked less than 100 cigarettes in their lifetime and ever smokers otherwise. Ever smokers were further classified as regular smokers (63% of controls) if they smoked one cigarette per day for 6 months or longer and occasional smokers (8% of controls) otherwise. Of the regular smoker controls, 37% were current smokers (i.e. they smoked within a year of the reference date) and 63% former smokers. We evaluated the potential interaction of pack-years of smoking as a continuous variable.

Genotyping

DNA for genotype assays was extracted from leukocytes using the Puregene® DNA Isolation Kit (Gentra Systems, Minneapolis, MN) for most cases ($n = 1{,}107$) and controls ($n = 1{,}032$) included in the analysis. DNA from an additional 43 cases and 117 controls was extracted from mouthwash samples using the phenol-chloroform extraction protocol.

Table 1 lists all SNPs determined using TaqMan (Applied Biosystems, Foster City, CA) or GoldenGate (Illumina®, San Diego, CA) assays. Genotyping was carried out at the core genotyping facility (CGF) of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, and description and methods for assays can be found at http://www.snp500cancer.nci.nih.gov (Packer et al. 2006). Initially, we selected 19 SNPs in 12 BER genes with expected minor allele

Table 1 Demographic and tumor characteristics of the study population

	Cases (n = 1,150)	Controls (n = 1,149)
Age[a]		
Mean age (SD), years	66 (10)	65 (10)
Gender[a]		
Male	1,004 (87)	1,002 (87)
Smoking status[b]		
Never	159 (14)	338 (29)
Occasional	50 (4)	88 (8)
Regular		
Former	474 (41)	458 (40)
Current	467 (41)	265 (23)
Stage at diagnosis		
Ta/LPMN	711 (62)	
T1	135 (12)	
T2	142 (12)	
T3	63 (5)	
T4	54 (5)	
Grade of differentiation		
G1	309 (28)	
G2	324 (29)	
G3	431 (39)	
LMPN	41 (4)	

Unless otherwise stated, data are number of participants and (%)

T, tumor stage; *G*, nuclear grade; *LMPN* low malignant potential neoplasms

[a] Matching factors by design

[b] Never smokers are those that smoked less than 100 cigarettes in their lifetime. Ever smokers were further classified as regular smokers if they smoked one cigarette per day for 6 months or longer and occasional smokers otherwise. Current smokers were those that smoked within a year of the reference date

frequency (MAF) >0.05 in Caucasians and with TaqMan assays available at the CGF. SNP selection favored non-synonymous SNPs, those previously evaluated in relation to bladder cancer risk, or those with evidence of functional significance. We later added 24 SNPs from a large-scale evaluation of candidate genes using the GoldenGate assay (García-Closas et al. 2006b). Due to low DNA amounts available at the time of analysis, 64 of the 1,150 cases and 116 of the 1,149 controls in this report were not included in the GoldenGate assays.

All assays were performed using randomly sorted DNA samples from cases and controls, including blinded duplicate samples for quality control. Duplicate quality control DNA samples (up to 93 pairs) displayed >98% agreement for all assays except for 96% agreement for the intronic SNP *PARP1* rs1805407, and 95% agreement for *XRCC1* rs25496 (V72A) and *XRCC1* rs25487 (Q399R). In addition, a subset of 6 SNPs (*APEX1* rs3136820, *PARP1* rs1136410, *PARP4* rs13428, *XRCC1* rs25487, *PCNA* rs25406, and *LIG3* rs1052536) were analyzed in both the GoldenGate and TaqMan assays and concordance was ≥99%.

All genotypes studied were in Hardy-Weinberg equilibrium (HWE) in the control population, except for *OGG1* rs125701, *PARP1* rs1805407 and *PARP4* rs750771, of which we observed slightly more homozygous variants than expected (3.5% observed versus 2.6% expected, $P = 0.04$, for *OGG1*; 4.1% observed and 3.1% expected, $P = 0.02$, for *PARP1*; and 5.7% observed and 4.5% expected, $P = 0.02$, for *PARP4*). Genotype concordance for these SNPs was 100%, thus these departures are unlikely to reflect genotyping errors. In addition, the number of assays with significant departures from HWE in this and previous reports from the same study population (García-Closas et al. 2005, 2006a, b) is no larger than expected by chance, indicating that the control population is in HWE, and that observed departures are likely to be due to chance.

Pairwise linkage disequilibrium (LD) between SNPs was estimated based on D' and r^2 values using Haploview (http://www.broad.mit.edu/mpg/haploview/index.php) (Stephens et al. 2001). Of the 43 SNPs, 3 were very rare (MAF < 0.01) in the study population (*PARP3* rs323870 (Q269R), *XRCC1* rs25496 (V72A), and *LIG3* rs3136025 (R780H)), and thus results are not reported. Four pairs of SNPs were highly correlated ($r^2 > 0.95$): *PARP1* rs1805415 and rs1136410 (V762A); *PARP1* rs747657 and rs1805414; *PARP1* rs747659 and rs1805407; and *POLB* rs2953983 and rs2979895. To reduce redundancy, we only show data from one of the two SNPs in these 4 correlated pairs.

Statistical analysis

For each individual SNP, we estimated odds ratios (OR) and 95% confidence intervals (95%CI) using logistic regression models adjusting for gender, age at interview in 5-year categories, region, and smoking status (never, occasional, former, and current). A global test for the association of genetic variation in the BER pathway and bladder cancer risk was tested using a likelihood ratio test (LRT) comparing the 36 individual SNPs analyzed in this study. Haplotype frequencies for genes with more than one SNP were estimated using HaploStats (version 1.2.1; http://www.mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm). We evaluated the robustness of our results using the false discovery rate (FDR). False discovery rate is the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypothesis among all the SNPs analyzed in the report. The FDR method was applied to the P-value for trend for each of the 36 non-redundant SNPs ($r^2 < 90\%$) evaluated in this report. Rather than using an arbitrary threshold FDR value,

we report the values for the most significant associations to allow the reader to evaluate the robustness of our findings. The Benjamini and Hochberg method was used to calculate FDR values using the program multtest in *R* (Benjamini 1995).

A meta-analysis was performed to summarize our findings along with previously published studies for the association between the *XRCC1* rs25487 (Q399R) polymorphism and bladder cancer risk. Peer reviewed studies published by October 2006 in English were identified using a *PubMed* search. Crude OR's and 95% CI's estimates were calculated using published genotype frequencies of cases and controls. A random-effect model was used to estimate summary OR's and 95%CI's by weighing each study result by a factor within- and between-study variance (Laird and Mosteller 1990). Homogeneity of study results was assessed by the *Q* test and publication bias by Begg's test (Begg and Mazumdar 1994) and Egger's test (Egger et al. 1997). Unless otherwise specified, statistical analyses were performed with STATA Version 9.1, special edition (STATA Corporation, College Station, TX).

Results

The study population was mostly males, and former or current smokers, and cases were predominantly diagnosed at early stages (Table 1). The 12 BER repair genes and 43 SNP's examined in this study along with MAFs in the control population are listed in Table 2. Because of very low MAFs or very high pairwise correlations, results from 7 of the 43 SNPs are not shown (see Methods for details; supplementary Table 1). Results for eight selected SNPs that had been previously evaluated in relation to bladder cancer risk, or had significant associations with bladder cancer risk in the current study, are shown in Table 3. The most significant association with bladder cancer risk was for a promoter SNP (rs125701) in the 8-oxoG DNA glycosylase gene (*OGG1*). Compared to individuals with the GG genotype, those with the GA or AA genotypes had significantly decreased risk for bladder cancer (OR (95%CI) 0.78 (0.63–0.97) and 0.74 (0.43–1.25) respectively; *P* for trend = 0.02; Table 3). The decreased risk for carriers of the *OGG1* rs125701 variant allele was stronger in current smokers [0.71 (0.49–1.03)], weaker in former smokers [0.82 (0.60–1.12)], and weakest in never smokers [0.93 (0.56–1.53)]. However, the genotype-smoking interaction was not statistically significant ($P = 0.41$ for current versus never). We found no significant interaction with pack-years of smoking (data not shown). Genotype frequencies for this SNP showed a small departure from HWE in the control population ($P = 0.04$, see Methods).

Variants in exon 7 of the modifying enzyme *PARP1* (rs1136410, V762A) and intron 1 of the BER DNA polymerase *POLB* (rs3136717) were associated with increases in bladder cancer risk; however, the increases in risk were observed for heterozygote individuals and not for homozygote variants (Table 3). The frequency of homozygote variant individuals for these two variants was small (2 and 1%, respectively), and when combined with heterozygote individuals and compared to common homozygotes, the association with bladder cancer risk maintained statistical significance [1.24 (1.02–1.51) and 1.30 (1.04–1.62), POLB (rs3136717) or PARP1 (rs1136410, V762A) respectively]. There was no significant evidence for an interaction with smoking status (never, former, current) or pack-years, for *POLB* (rs3136717) or *PARP1* (rs1136410, V762A).

Data suggested that the association with *PARP1* V762A and bladder cancer risk might be limited or stronger among older individuals (age-specific OR (95%CI) 1.33 (1.08–1.64) for subjects >60 years of age, and 0.79 (0.55–1.12); the *P* for interaction assuming a linear relationship with age was 0.004.

Analysis of pairwise joint associations with the BER variants noted above suggested an interaction between *POLB* rs3136717 and *PARP1* rs1136410 (V762A) (*P*-interaction = 0.09), with a joint OR (95%CI) for those individuals who carried both variants compared to those with common alleles of 1.93 (1.29–2.88) (Table 4). No evidence of effect modification by cigarette smoking, pack years of smoking, age, or gender was found for any of the other SNPs analyzed. Furthermore, haplotypes in genes with multiple variants did not reveal significant associations with bladder cancer risk beyond those observed in individual analysis (supplementary Table 2).

We evaluated our results using the FDR approach as described in the Methods. FDR values for the three most significant findings were 0.53 for *OGG1* rs125701, 0.97 for *PARP1* rs1136410 (V762A) and 0.81 for *POLB* rs3136717, taking into account all non-redundant SNP's evaluated in this report. This indicated that there is a substantial chance that these findings are false positives, and thus need to be confirmed in future studies. A global test for the 36-selected BER pathway SNPs analyzed, did not show a significant overall association with bladder cancer risk (global LRT (36 *df*) $P = 0.62$).

Meta-analysis

Previous reports suggested reduced bladder cancer risk among individuals homozygote for the variant *XRCC1*

Table 2 Description of 43 single nucleotide polymorphisms in 12 base excision DNA repair genes evaluated in the Spanish Bladder Cancer Study

Gene symbol and name		Chromosomal location	dbSNP ID	Nucleotide change	Amino acid change, or UTR	Genotyping assay	Minor allele frequency in control population
OGG1	8-Oxoguanine DNA glycosylase	3p26.2	rs125701	−1492G > A	5′UTR	GoldenGate	0.16
			rs1052133	Ex6−315C > G	S326C	TaqMan	0.24
			rs2304277	IVS7 + 110G > A		GoldenGate	0.23
MUTYH	MutY homolog (*E. coli*) DNA glycosylase	1p34.3–p32.1	rs3219466	Ex1 + 8 > C > T	5′UTR	TaqMan	0.02
APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1	14q11.2–q12	rs1760944	−655T > G	5′UTR	GoldenGate	0.36
			rs3136814	Ex1 + 8A > C		GoldenGate	0.04
			rs3136820	Ex5 + 5T > G	D148E	TaqMan	0.47
PARP1	Poly (ADP-ribose) polymerase family, member 1	1q41–q42	rs1805407	IVS2 + 82A > G		GoldenGate	0.17
			rs1805414	Ex7 + 18T > C		GoldenGate	0.30
			rs1805415	Ex8 + 45G > A		GoldenGate	0.12
			rs1136410	Ex17 + 8T > C	V762A	TaqMan	0.12
			rs747657	IVS20−63G > C		GoldenGate	0.30
			rs747659	IVS21 + 59G > A		GoldenGate	0.17
PARP3	Poly (ADP-ribose) polymerase family, member 3	3p21.31–p21.1	rs323870	Ex6−56A > G	Q269R	TaqMan	0.001
PARP4	Poly (ADP-ribose) polymerase family, member 4	13q11	rs1539096	Ex3−89A > G		GoldenGate	0.42
			rs1807111	IVS15−995C > T		GoldenGate	0.09
			rs750771	IVS17−110G > A		GoldenGate	0.21
			rs6413414	Ex20−19A > T		GoldenGate	0.09
			rs1050110	Ex31 + 128G > C	G1265A	TaqMan	0.34
			rs13428	Ex31 + 172G > C	G1280R	TaqMan	0.32
			rs1050112	Ex31 + 316C > A	P1328T	TaqMan	0.33
			rs1050114	Ex31 + 325G > A	A1331T	TaqMan	0.01
			rs7571	Ex33−14G > C	A1656P	TaqMan	0.33
XRCC1	X-ray repair complementing defective repair in Chinese hamster cells 1	19q13.2	rs25496	Ex3−41T > C	V72A	TaqMan	0.002
			rs1799782	Ex6−22C > T	R194W	TaqMan	0.06
			rs25489	Ex9 + 16G > A	R280H	TaqMan	0.05
			rs25487	Ex10−4G > A	Q399R	TaqMan	0.34
POLB	Polymerase (DNA directed), beta	8q43	rs3136717	IVS1−89T > C		GoldenGate	0.10
			rs2979895	IVS2−2264A > G		GoldenGate	0.07
			rs2953983	IVS7 + 171A > G		GoldenGate	0.07
POLD1	Polymerase (DNA directed), delta 1, catalytic subunit 125 kDa	19p15.3–p15.2	rs1673026	IVS2 + 21T > C		GoldenGate	0.08
PCNA	proliferating cell nuclear antigen	20q31.3	rs25406	IVS2−124C > T		TaqMan	0.42
			rs17349	IVS2−4C > T		GoldenGate	0.13
			rs17352	IVS5 + 140A > C		GoldenGate	0.14
LIG1	Ligase I, DNA, ATP-dependent	19q13.2–q13.3	rs20579	Ex2−24C > T		GoldenGate	0.14
			rs20580	Ex7 + 44C > A		GoldenGate	0.49
			rs156641	IVS19−131G > A		GoldenGate	0.36
			rs3729512	IVS25 + 19G > A		GoldenGate	0.11
			rs13436	Ex26 + 3G > C		GoldenGate	0.45
LIG3	Ligase III, DNA, ATP-dependent	17q11.2–q12	rs3136025	Ex18−75G > A	R780H	TaqMan	0.002
			rs3136027	IVS18−148A > T		TaqMan	0.05
			rs2074522	IVS18−39G > A		TaqMan	0.08
			rs1052536	Ex21−250C > T	3′UTR	TaqMan	0.40

Table 3 Association of 8 selected polymorphisms in 5 base excision DNA repair genes and bladder cancer risk, adjusted for gender, age, region and smoking status (1,150 cases and 1,149 controls)

Gene	Polymorphism	Genotype	Cases		Controls				
			N	%	*N*	%	OR	95% CI	
OGG1	rs125701	GG	790	76	688	71	1.00		
	−1492G > A	AG	220	21	246	25	0.78	0.63	0.97
		AA	28	3	34	4	0.74	0.43	1.25
							P for trend =		0.02
		AG or AA					0.78	0.63	0.96
	rs1052133	*CC*	649	60	596	59	1.00		
	Ex6−315C > G (S326C)	*CG*	383	35	361	35	0.93	0.77	1.12
		GG	56	5	61	6	0.84	0.57	1.26
							P for trend =		0.30
APEX1	rs3136820	TT	335	31	292	29	1.00		
	Ex5 + 5T > G (D148E)	TG	510	47	491	48	0.90	0.73	1.11
		GG	249	23	230	23	0.89	0.69	1.14
							P for trend =		0.32
PARP1	rs1136410	TT	825	72	873	77	1.00		
	Ex17 + 8T > C (V762A)	TC	294	26	235	21	1.28	1.04	1.58
		CC	19	2	23	2	0.80	0.42	1.53
							P for trend =		0.10
		TC or CC					1.24	1.02	1.51
XRCC1	rs1799782	CC	967	88	906	89	1.00		
	Ex6−22C > T (R194W)	CT	124	11	115	11	0.98	0.74	1.29
		TT	5	1	1	0.1	–	–	–
		CT or TT					1.01	0.76	1.33
	rs25489	GG	955	88.3	911	90	1.00		
	Ex9 + 16G > A (R280H)	GA	122	11.3	101	10	1.08	0.80	1.44
		AA	4	0.4	4	0.4	1.00	0.23	4.36
							P for trend =		0.64
		GA or AA					1.07	0.81	1.43
	rs25487	GG	434	41	433	43	1.00		
	Ex10−4G > A (Q399R)	GA	494	47	453	45	1.10	0.91	1.33
		AA	133	13	110	11	1.15	0.86	1.56
							P for trend =		0.25
POLB	rs3136717	TT	829	76	831	80	1.00		
	IVS1−89T > C	TC	244	22	188	18	1.34	1.07	1.67
		CC	13	1	14	1	0.83	0.37	1.84
							P for trend =		0.05
		TC or CC					1.30	1.04	1.62

SNPs were selected because they had significant associations with risk in the Spanish Bladder Cancer Study or were previously evaluated in relation to bladder cancer. Differences between total number of cases and controls and numbers in table are due to missing genotype data

rs25487 (Q399R) polymorphism (a total of 1,801 cases and 1,898 controls; summary OR (95%CI) 0.90 (0.72–1.11)) (Kelsey et al. 2004; Matullo et al. 2001; Sanyal et al. 2004; Shen et al. 2003; Stern et al. 2001; Wu et al. 2006). Inclusion of data from the Spanish Bladder Cancer Study resulting in a substantial increase in total sample size (2,862 cases and 2,894 controls in total) did not show evidence for a significant reduction in risk [summary OR (95%CI) 0.99 (0.83–1.19); Fig. 1]. The frequency of homozygote variant individuals in the control population from the Spanish study (11%) was similar to that reported in previous studies (ranging from 9 to 14%), and there was no evidence for study heterogeneity or publication bias, according to the *Q* test, and the Eggers' and Begg's tests, respectively.

Discussion

Our investigation of 43 single nucleotide polymorphisms (SNPs) in 12 BER genes in a large case-control study of bladder cancer risk, found no overall association with bladder cancer risk (global LRT (36 *df*) $P = 0.62$). However, data suggested associations with specific SNPs in three genes, *OGG1*, *PARP1* and *POLB*.

A variant (rs125701) in the promoter region of *OGG1* was inversely associated with bladder cancer risk in our study population. *OGG1* is involved in the removal of 8-oxoG lesions (oxidized guanine bases) resulting from exposure to ROS. If not removed by BER, these lesions can result in an increase in G to T

Table 4 Pairwise joint associations for three selected BER SNPs *OGG1* rs125701, *PARP1* rs1136410 (V762A), and *POLB* rs3136717, and bladder cancer risk adjusted for region, gender, age and smoking status

Genotype 1	Genotype 2	cases	control	OR	95% CI		*P*	*P* interaction
OGG1 rs125701 (−1492G > A)	*PARP1* rs1136410 (Ex17 + 8T > C, V762A)							
AA	*TT*	23	23	1.00				
GA or *GG*	*TT*	727	718	1.00	0.54	1.84	0.99	
AA	*TC* or *CC*	5	9	0.55	0.15	2.00	0.37	
GA or *GG*	*TC* or *CC*	273	208	1.23	0.65	2.31	0.52	0.23
OGG1 rs125701 (−1492G > A)	*POLB* rs3136717 (IVS1−89C > T)							
AA	*CC*	20	28	1.00				
GA or *GG*	*CC*	778	748	1.37	0.75	2.51	0.31	
AA	*CT* or *TT*	8	6	1.60	0.46	5.55	0.46	
GA or *GG*	*CT* or *TT*	232	186	1.67	0.89	3.13	0.11	0.67
PARP1 rs1136410 (Ex17 + 8T > C, V762A)	*POLB* rs3136717 (IVS1−89C > T)							
TT	*CC*	607	632	1.00				
TC or *CC*	*CC*	213	189	1.09	0.86	1.39	0.45	
TT	*CT* or *TT*	174	157	1.14	0.88	1.48	0.31	
TC or *CC*	*CT* or *TT*	81	45	1.93	1.29	2.88	0.001	0.09

SNPs were selected because they had significant association with risk in the Spanish bladder cancer study

transversion mutations (Dhenaut et al. 2000), which could influence the risk of developing cancer. Although the functional significance of the *OGG1* rs125701 SNP is unknown, evaluation of the functional significance of this variant, as it has been performed for other variants in BER proteins (Sokhansanj and Wilson 2006), would be useful in interpreting potential associations. Our data suggested that the inverse risk association was strongest among current smokers and weakest among never smokers. Although the modification of the genotype association by smoking was not statistically significant, this observation is consistent with previous studies showing that levels of 8-oxoG and repair activity for 8-oxoG are higher in smokers compared to non-smokers (Asami et al. 1996, 1997). In addition to the promoter SNP, the *OGG1* rs1052133 (S326C) has been reported on in one previous study (Wu et al. 2006) and suggested decreased bladder cancer risk among heterozygote and homozygote variants, which is supported by our results.

PARP1 belongs to a family of enzymes that, through consumption of NAD+, conjugates ADP-ribose polymers to a variety of target proteins (including *XRCC1*), and this modification is important for proper DNA repair function (Vidakovic et al. 2005). Our data suggested an association with a non-synonymous SNP in *PARP1* rs1136410 (V762A) and increased bladder cancer risk. A previous study demonstrated that this polymorphism is associated with decreased enzymatic activity using freshly isolated peripheral lymphocytes from cancer-free subjects, and increased prostate cancer risk (Lockett et al. 2004). However, a previous report on this SNP suggested decreased bladder cancer risk (Wu et al. 2006). Future studies will be needed to clarify if this putative functional SNP is associated with bladder cancer risk.

POLB encodes the major gap-filling enzyme for BER and interacts directly with BER proteins including LIG1, APE1, PARP1, XRCC1 and PCNA. *POLB* is mapped to chromosome region 8p a region that is deleted in about 25% of bladder cancers (Knowles et al. 1993), which suggests that it may play an important role in bladder carcinogenesis. We found an association with bladder cancer risk with an intronic SNP (rs3136717) in the *POLB* gene of which the functional implications are unknown. Furthermore, analysis of pairwise joint associations suggested an interaction between *PARP1* rs1136410 and *POLB* rs3136717 variants (P for interaction = 0.09), resulting in an almost twofold increase in risk for subjects carrying variants for these two SNPs. It has been shown that *PARP1* can influence the activity of *POLB* in in vitro assays of BER (Prasad et al. 2001), and thus a biological interaction between these genes is plausible; however, future studies are needed to confirm their potential joint or independent associations with bladder cancer risk.

Bladder cancer risk and *XRCC1* genetic variation has been the focus of prior epidemiological studies (Andrew et al. 2005; Kelsey et al. 2004; Matullo et al. 2001; Sanyal et al. 2004; Shen et al. 2003; Stern et al. 2001; Wu et al. 2006). The *XRCC1* gene encodes the major coordinating protein of BER and interacts with PARP1, LIG3 and POLB. The *XRCC1* rs25487 (Q399R) variant results in an amino acid substitution

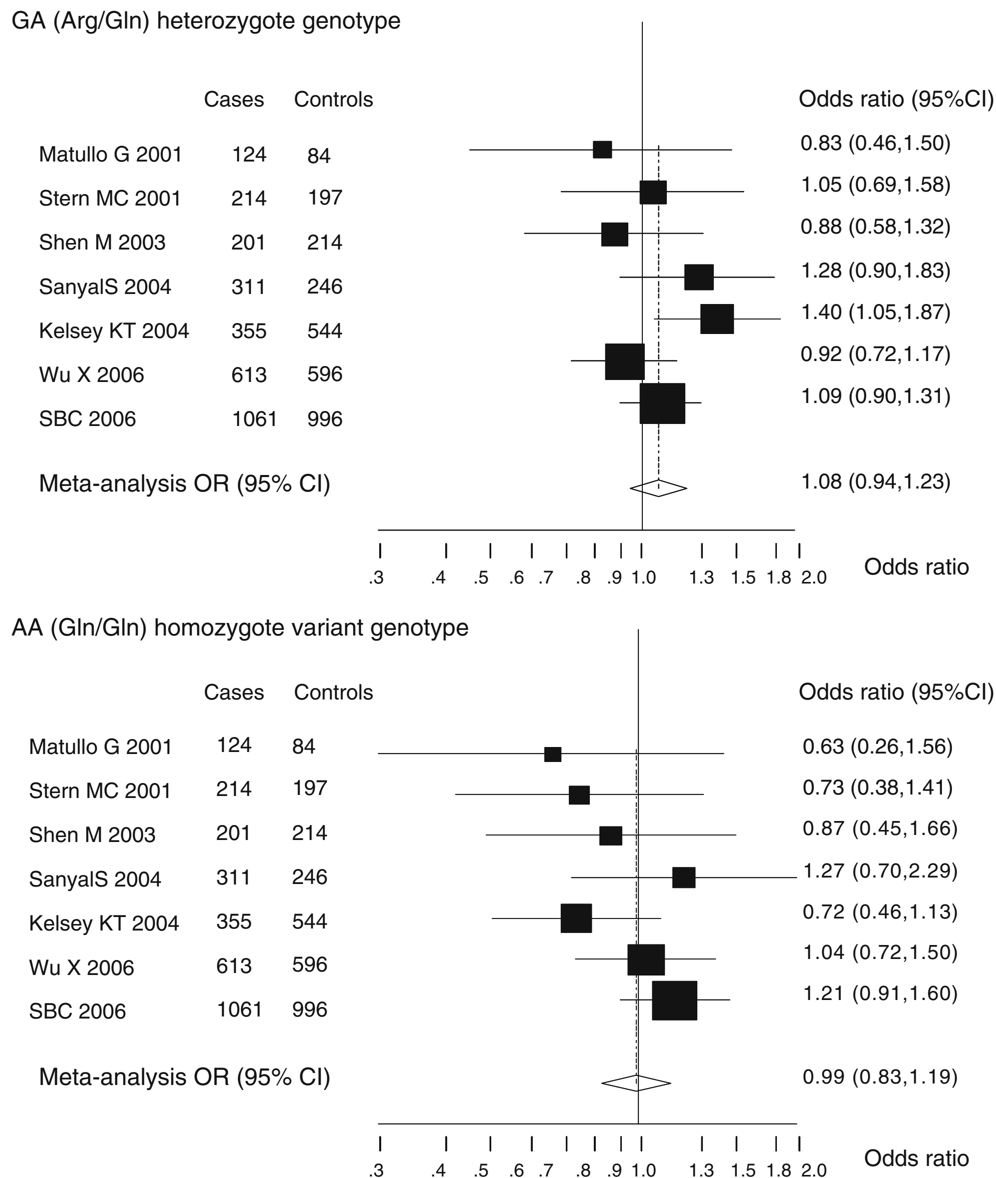

Fig. 1 Meta-analysis of the association of *XRCC1* rs25487 Ex10-4 G > A (Arg399Gln) and bladder cancer risk reported in this and previously published studies

in the region of *XRCC1* responsible for interacting with PARP1 (Wang et al. 2003). However, meta-analyses including a total 2,900 cases and 2,893 controls from our study population and six previously published studies (Kelsey et al. 2004; Matullo et al. 2001; Sanyal et al. 2004; Shen et al. 2003; Stern et al. 2001; Wu et al. 2006) showed no significant overall association with bladder cancer risk 1.08 (0.94–1.23) and 0.99 (0.83–1.19) for heterozygote and homozygote variants, respectively. We found no significant evidence for modification by pack years of smoking, or smoking status as was previously suggested (Matullo et al. 2001; Stern et al. 2001). In addition, analyses of data from our study showed no evidence for interactions with variants in *PARP1*, *LIG3* and *POLB*.

From the SNPs evaluated in this report, two other variants in *XRCC1* and one variant in *APEX* have been previously evaluated with respect to bladder cancer risk. Associations with bladder cancer risk for CT/TT genotypes for a relatively rare non-synonymous polymorphism in *XRCC1* rs1799782 (R194W) have been inconsistent compared to the CC genotype in three US studies (Andrew et al. 2005; Stern et al. 2001; Wu et al. 2006), and this SNP was not associated

with risk in our large study population. A previous study suggested modification of this association by pack-years (Stern et al. 2001), however our analysis did not support these findings. Lastly, two reports suggested a decrease in bladder cancer risk among homozygous variants for the *APEX1* rs3136820 (D148E) SNP, (Andrew et al. 2005; Wu et al. 2006) and our data was consistent with these previous findings.

Strengths of our study population included a large sample size with high participation rates. To our knowledge, this study represents the most comprehensive analysis of genetic variation in BER performed to date in bladder cancer. However, because we chose SNPs prioritized by potential functional significance rather than selecting SNPs to capture most of the common genetic variation in the genes evaluated, additional polymorphisms in BER genes not captured by our analyses could be associated with bladder cancer risk. Furthermore, we cannot exclude the possibility that the observed associations are false positive findings based on FDR calculations which indicated that findings are not robust. Further studies are thus required to confirm suggested associations.

In summary, meta-analyses indicated that *XRCC1* rs25487 (Q399R), which has been the focus of previous studies on BER variants and bladder cancer risk, is unlikely to be related to risk. However, our data suggested bladder cancer risk associations with specific SNPs in *OGG1*, *PARP1* and *POLB*, which need to be confirmed in independent study populations.

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Appendix: Participating study centers in Spain

Institut Municipal d'Investigació Mèdica, Universitat Pompeu Fabra, Barcelona–Coordinating Center (M. Kogevinas, N. Malats, F.X. Real, F Fernandez, M. Sala, G. Castaño, M. Torà, D. Puente, C. Villanueva, C. Murta, J. Fortuny, E. López, S. Hernández, R. Jaramillo); Hospital del Mar, Universitat Autònoma de Barcelona, Barcelona (J. Lloreta, S. Serrano, L. Ferrer, A. Gelabert, J. Carles, O. Bielsa, K. Villadiego), Hospital Germans Tries i Pujol, Badalona, Barcelona (L. Cecchini, J.M. Saladié, L. Ibarz); Hospital de Sant Boi, Sant Boi, Barcelona (M. Céspedes); Centre Hospitalari Parc Taulí, Sabadell, Barcelona (C. Serra, D. García, J. Pujadas, R. Hernando, A. Cabezuelo, C. Abad, A. Prera, J. Prat); ALTHAIA, Manresa, Barcelona (M. Domènech, J. Badal, J. Malet); Hospital Universitario, La Laguna, Tenerife (R. García-Closas, J. Rodríguez de Vera, A.I. Martín); Hospital La Candelaria, Santa Cruz, Tenerife (J. Taño, F. Cáceres); Hospital General Universitario de Elche, Universidad Miguel Hernández, Elche, Alicante (A. Carrato, F. García-López, M. Ull, A. Teruel, E. Andrada, A. Bustos, A. Castillejo, J.L. Soto); Universidad de Oviedo, Oviedo, Asturias (A. Tardón); Hospital San Agustín, Avilés, Asturias (J.L. Guate, J.M. Lanzas, J. Velasco); Hospital Central Covadonga, Oviedo, Asturias (J.M. Fernández, J.J. Rodríguez, A. Herrero), Hospital Central General, Oviedo, Asturias (R. Abascal, C. Manzano); Hospital de Cabueñes , Gijón, Asturias (M. Rivas, M. Arguelles); Hospital de Jove, Gijón, Asturias (M. Díaz, J. Sánchez, O. González); Hospital de Cruz Roja, Gijón, Asturias (A. Mateos, V. Frade); Hospital Alvarez-Buylla (Mieres, Asturias): P. Muntañola, C. Pravia; Hospital Jarrio, Coaña, Asturias (A.M. Huescar, F. Huergo); Hospital Carmen y Severo Ochoa, Cangas, Asturias (J. Mosquera).

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