DNA Repair Gene *ERCC2, XPC, XRCC1, XRCC3* Polymorphisms and Associations with Bladder Cancer Risk in a French Cohort

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Abstract. *In polygenic diseases, association studies look for genetic variation such as polymorphisms in low penetrance genes, i.e. genes in interaction with environmental factors. DNA repair systems that protect the genome from deleterious endogenous and exogenous damage have been shown to significantly reduce activity. In particular, enzymes of the nucleotide excision repair pathway are suspected to be implicated in cancer. In this study bladder cancer which is viewed as a polygenic disease was investigated. The functional polymorphisms of four DNA repair genes, excision repair cross-complementing group 2 (ERCC2), Xeroderma Pigmentosum group C (XPC), and X-ray repair cross-complementing groups 1 and 3 (XRCC1 and XRCC3) were analyzed. The studied population included 51 bladder cancer cases and 45 controls. The genotyping of six SNP (single nucleotide polymorphism) was carried out on these populations with the MGB (Minor Groove Binder) probe technique which uses allelic discrimination with the Taqman® method. The Gln allele of the XPC 939 polymorphism was found to be associated with an increase in bladder cancer risk.*

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Urinary bladder cancer is one of the most common types of neoplasm in men in Western Europe (1). Cigarette smoke, containing several potent chemical carcinogens, is the predominant risk factor and occupational exposure to carcinogens, especially to some aromatic amines and to some polycyclic aromatic hydrocarbons, is the second major risk factor in Western countries (2, 3).

Exposure to these chemical carcinogens may lead to DNA damage, contributing to neoplastic transformation, but various biological mechanisms respond to repair the DNA damage (4). Although many people are exposed to the risk factors, only a fraction of exposed individuals will develop bladder cancer, suggesting an individual susceptibility to the effects the carcinogens.

Variation in the expression or functionality of DNA repair genes, caused by genetic polymorphisms that lead to amino acid substitution, may influence an individual's capacity to repair DNA damage and thus might contribute to this variation (5). Polymorphisms in some DNA repair genes have been evaluated as risk factors in bladder cancer in a number of epidemiological studies. However, to date, studies of bladder cancer risk in relation to DNA repair gene polymorphisms have been rare and often conflicting. Moreover, to the best of our knowledge, no French studies on this topics have been published.

Therefore, in the present study, a hospital-based case control study of French Caucasian men was performed and six SNP were analysed to investigate the association between polymorphisms of four DNA repair genes involved in different repair pathways, with bladder cancer risk.

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Table I. *Six functional polymorphisms were studied in four DNA repair genes (ERCC2, XPC, XRCC1 and XRCC3)*

Gene symbols	Names	Studied polymorphisms (SNP)	Polymorphism NCBI references
ERCC2/XPD	Excision repair cross-complementing group 2	*Asp312Asn* (G23591A (exon 10))	rs1799793
	Xeroderma Pigmentosum group D	*Lys751Gln* (A35931C (exon 23))	rs13181
XPC	Xeroderma Pigmentosum group C	*Lys939Gln* (A2920C (exon 15))	rs2228001
XRCC1	X-ray repair cross-complementing group 1	*Arg194Trp* (C26304T (exon 6))	rs1799782
		Arg399Gln (G28152A (exon 10))	rs25487
XRCC3	X-ray repair cross-complementing group 3	*Thr241Met* (C18067T)	rs861539

SNP: single nucleotide polymorphism.

Patients and Methods

Study subjects. Recruitment was carried out among men hospitalized in the urology department of the CHU (University Hospital Center) of Clermont-Ferrand from June 2004 to April 2005. The cases were newly diagnosed bladder cancer patients with histological confirmation by the anatomopathologist and the controls were patients admitted to the same hospital during the same period of time, with urological non-neoplastic diseases. All the study subjects received counselling and provided written consent for the study. The included subjects (51 cases and 45 controls) were all Caucasians.

Genotyping. For patients and controls, Whatman FTA® cards (Whatman Inc, Florham Park, USA) were chosen for blood sample collection. The cards were archived and the elution of genomic DNA was performed at room temperature according to the Whatman FTA procedure.

Six functional polymorphisms of four DNA repair genes (*ERCC2, XPC, XRCC1* and *XRCC3*) were selected (Table I). The corresponding probes were obtained from Applied Biosystems (Foster City, CA, USA). Allelic discrimination using fluorogenic probes (5' nuclease assay, Taqman®) was chosen for genotyping on the ABI PRISM 7700 Sequence Detection System (Applied Biosystems), and consisted of the use of allele-specific fluorogenic probes (6). The primer and probe sequences were synthesised by Applied Biosystems (Cheshire, UK). Sixteen nanograms of DNA were amplified by AmpliTaq Gold DNA polymerase which was included in the Taqman® Universal Master Mix (Applied Biosystems, Foster City). The PCR reactions were as follows: one step of 10 min at 95˚C followed by 40 cycles of two-step PCR with denaturation at 92˚C for 15 sec and annealing and extension at 60˚C for 1 min. Ten percent of all the samples were genotyped again for quality control.

Statistical analysis. The software SEM (created by Centre Jean Perrin, Clermont Ferrand, France) was used for data analyses (7). The standard descriptive statistics consisted of means plus standard deviation for the quantitative data. Comparisons between cases and controls were performed using the Chi-square test for the qualitative parameters. Univariate and multivariate analyses were performed to determine the cancer risk factors in our populations, and odds ratios (ORs) with corresponding 95% confidence interval (95% CI) were estimated. The allelic frequencies and the distribution of genotypes were compared within the two populations using Chi-square analysis and ORs with 95% CI. A stratified analysis on smoking status was performed for significant ORs.

Table II. *ERCC2 Asp312Asn, ERCC2 Lys751 Gln, XPC Lys939Gln, XRCC1 Arg194Trp, XRCC1 Arg399Gln, XRCC3 Thr241Met polymorphisms and bladder cancer risk.*

Genotype	Cases (%)	Controls (%)	OR* (95% CI)
ERCC2 Asp312Asn			
Asp/Asp	25 (49)	21 (46)	1.00 (reference)
Asp/Asn	19 (37)	18 (40)	0.91 (0.51-1.64)
Asn/Asn	7 (13)	6 (13)	0.83 (0.26-2.69)
ERCC2 Lys751Gln			
Lys/Lys	11 (22)	6 (13)	1.00 (reference)
Lys/Gln	18 (35)	17 (38)	0.80 (0.46-1.39)
Gln/Gln	22 (43)	22 (49)	0.65 (0.21-1.94)
XPC Lys939Gln			
Lys/Lys	7 (13)	15 (33)	1.00 (reference)
Lys/Gln	22 (43)	24 (53)	2.69 (1.42-5.11)
Gln/Gln	22 (43)	6 (13)	7.24 (2.01-26.12)
XRCC1 Arg194Trp			
Arg/Arg	0 (0)	0 (0)	1.00 (reference)
Arg/Trp	4 (8)	5 (11)	1.50 (0.37-6.08)
Trp/Trp	47 (92)	40 (89)	2.25 (0.14-36.92)
XRCC1 Arg399Gln			
Arg/Arg	21 (41)	18 (40)	1.00 (reference)
Arg/Gln	25 (49)	18 (40)	0.75 (0.42-1.35)
Gln/Gln	5 (10)	9 (20)	0.56 (0.17-1.82)
XRCC3 Thr241Met			
Thr/Thr	8 (15)	4 (9)	1.00 (reference)
Thr/Met	28 (55)	23 (51)	0.62 (0.33-1.18)
Met/Met	15 (30)	18 (40)	0.38 (0.11-1.39)

*Adjusted on age in unconditional logistic regression.

Results

Risk associated with individual SNPs. There were no significant modifications of risk, except with the SNP of XPC, where the presence of the Gln allele was associated with a significant increase in the risk, after adjustment for age (Lys/Gln: OR=2.69 with 95% CI =1.42-5.11; Gln/Gln: OR=7.24 with 95% CI =2.01-26.12).

Interaction between genetic factors and tobacco smoking. The prevalence of smoking was higher among cases (data not

Table III. *XPC Lys939Gln polymorphisms and bladder cancer risk stratified by smoking*.

	Nonsmokers			Smokers		
Genotype	Cases (%)	Controls (%)	OR (CI 95%)	Cases (%)	Controls (%)	OR (CI 95%)
XPC Lys939Gln						
Lys/Lys	4 (12)	12 (32)	1.00 (reference)	3 (19)	2 (33)	1.00 (reference)
Lys/Gln	16 (47)	20 (53)	2.40 (0.66-8.72)	6 (38)	4 (67)	-
Gln/Gln	14 (41)	6 (16)	7.00 (1.69-29.00)	7 (44)	0 (0)	-

shown). The genotype and allele frequencies of Asp312Asn ERCC2, Lys751Gln ERCC2, Lys939Gln XPC, Arg194Trp XRCC1, Arg399Gln XRCC1 and Thr241Met XRCC3 polymorphisms among the cases and controls, as well as their associations with risk of bladder cancer, are shown in Table II. Lys939Gln XPC heterozygote and homozygote polymorphisms were associated with a significantly increased risk of bladder cancer (Lys939Gln XPC heterozygote OR 2.69, CI 1.42-5.11; Lys939Gln XPC homozygote OR 7.24, 95% CI 2.01-26.12). The stratified analysis of smoking revealed the same association in the nonsmokers group with similar OR (Lys939Gln XPC heterozygote OR 2.4, CI 0.66-8.72; Gln/Gln: OR 7.00, 95% CI 1.69-29.00) (Table III); the number of smokers was too small to calculate the OR.

Discussion

Although our study had limited power, given the sample size, a significant increased risk for cases with the Lys939Gln *XPC* heterozygote and homozygote variants was found. The stratified analysis of smoking showed that the Lys939Gln *XPC* homozygote variant was associated with bladder cancer risk among the nonsmokers. The association between Lys939Gln *XPC* polymorphisms and bladder cancer risk in Caucasian or European populations has been investigated in only a few studies. Sanyal *et al*. found an increased risk for cases with the Lys939Gln homozygous variant whereas three other studies did not (8-11). Xeroderma pigmentosum group C (XPC) is an important DNA damage recognition protein that binds to damaged DNA at a very early stage during DNA repair. The XPC protein is also involved in DNA damage-induced cell cycle checkpoint regulation and apoptosis. XPC defects are probalbly associated with critical events in human bladder cancer carcinogenesis and progression (12).

The Arg399Gln *XRCC1* homozygote variant genotype was inversely associated with bladder cancer risk in the present study, whereas the Arg194Trp *XRCC1* genotype was associated with an increased risk, but these results were not significant. Several epidemiological studies have assessed bladder cancer risk and Arg399Gln *XRCC1* polymorphism (8, 13-18). Many of them have suggested a decreased risk for individuals with the variant homozygote genotype (Gln/Gln) (14-17). Three studies, involving mainly Caucasian subjects, provided no evidence of an association between Arg399Gln *XRCC1* polymorphism and bladder cancer risk (8, 18, 19). Finally, Figueroa *et al*. (20) carried out a meta-analysis including a total of 2,900 cases and 2,893 controls from their study (Spanish Bladder Cancer Study) and six previously published studies (8, 14-18) that showed no significant overall association with bladder cancer risk with an OR of 1.08 (0.94-1.23) and 0.99 (0.83-1.19) for heterozygote and homozygote variants, respectively. Moreover, they found no significant evidence for modification by pack years of smoking, or smoking status as had been previously suggested (15, 17).

Few investigations of the association between Arg194Trp *XRCC1* polymorphisms and bladder cancer risk have been conducted and no significant association has been found (13, 17, 18, 20). However a modest protective effect in some of these studies was suggested especially among smokers (13, 17, 18, 20). Finally, according to Stern *et al*. (17), the variant alleles at codons 194 and 399 each might have a protective effect in the absence of the other, suggesting that they might independently affect risk.

The Thn241Met *XRCC3* heterozyte and homozygote variant genotypes were associated with a decreased risk of bladder cancer in the present study, but these results were not significant. The study of Shen *et al*. (16) was the first and the only one to suggest a protective role of *XRCC3* codon 241 polymorphism against bladder cancer risk which was stronger among heavy smokers. This result was not reproduced, and contradictory results were obtained in two case control studies, for one restricted to non-smokers and ex-smokers and for the other especially for smokers (21, 22). Figueroa *et al*, in the meta-analysis cited above, observed a significant small increase in risk among homozygote variants (20). More recently, Andrew *et al*. (23) analysed these genotypes in two case-control studies of bladder cancer incidence, one conducted in New Hampshire, USA and the other in Turin, Italy (23), and they also observed an increased risk with the variant XRCC3-241 (TT) genotype, mainly among current smokers.

In the present study, the Gln751Gln and Lys751Gln *ERCC2/XPD* variants were associated with a decreased risk, but these results were not significant. Stern *et al*. (21) also found a small but nonsignificant decrease in risk for the

Gln/Gln genotype compared with subjects with the Lys/Lys or Lys/Gln genotypes whereas most of the published studies did not find any association between *ERCC2/XPD* polymorphisms and bladder cancer risk (11, 16, 24-26).

No significant association was observed in the present study between Asp312Asn *ERCC2/XPD* polymorphisms and bladder cancer risk.

Conclusion

Genetic polymorphisms of Lys939Gln *XPC* may be associated with an individual susceptibility to bladder cancer. This study provides no evidence of association between *ERCC2* (Asp312Asn, Lys751Gln), *XRCC1* (Arg194Trp Arg399Gln) and *XRCC3* (Thr241Met) polymorphisms and bladder cancer risk. Additional studies on Lys939Gln *XPC* polymorphisms are required to confirm or refute the finding of this study.

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