Research article

DNA repair gene XRCC1 polymorphisms and bladder cancer risk

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Abstract

Background: Cigarette smoking and chemical occupational exposure are the main known risk factors for bladder transitional cell carcinoma (TCC). Oxidative DNA damage induced by carcinogens present in these exposures requires accurate base excision repair (BER). The XRCC1 protein plays a crucial role in BER by acting as a scaffold for other BER enzymes. Variants in the XRCC1 gene might alter protein structure or function or create alternatively spliced proteins which may influence BER efficiency and hence affect individual susceptibility to bladder cancer. Recent epidemiological studies have shown inconsistent associations between these polymorphisms and bladder cancer. To clarify the situation, we conducted a comprehensive analysis of 14 XRCC1 polymorphisms in a case-control study involving more than 1100 subjects.

Results: We found no evidence of an association between any of the 14 XRCC1 polymorphisms and bladder cancer risk. However, we found carriage of the variant Arg280His allele to be marginally associated with increased bladder cancer risk compared to the wild-type genotype (adjusted odds ratio [95% confidence interval], 1.50 [0.98–2.28], p = 0.06). The association was stronger for current smokers such that individuals carrying the variant 280His allele had a two to three-fold increased risk of bladder cancer compared to those carrying the wildtype genotype (p = 0.09). However, the evidence for gene-environment interaction was not statistically significant (p = 0.45).

Conclusion: We provide no evidence of an association between polymorphisms in XRCC1 and bladder cancer risk, although our study had only limited power to detect the association for low frequency variants, such as Arg280His.

Background

Bladder cancer is the fourth most common malignancy in Europeans [1]. Tobacco smoking and occupational exposure to chemicals are two well established risk factors [2,3]. Associated carcinogens damage DNA, and failure of accurate repair can result in mutations which may trigger carcinogenesis.

Interindividual variability in DNA repair capability (DRC) is an important factor influencing an individual’s cancer
risk [4]. DNA repair gene polymorphisms may contribute to this variation [5]. XRCC1 is an essential DNA repair gene involved in base excision repair (BER) [6]. Spontaneous chromosome aberrations and deletions are seen in XRCC1 mutant cells (EM9), and XRCC1 knock out (-/-) mice are embryonic lethal [7,8].

The XRCC1 gene exhibits polymorphic variations, including three common single nucleotide polymorphisms (SNPs) that result in amino acid substitutions in exon 7 (Arg194Trp), exon 9 (Arg280His) and exon 10 (Arg399Gln). These nonconservative amino acid alterations may influence DRC by altering the protein-protein interactions between XRCC1 and other BER proteins. The Arg399Gln variant was found to be associated with several phenotypic alterations, including higher levels of sister chromatid exchange [9], aflatoxin B1-DNA adducts, glyco-ophorin A mutations [10] and polyphenol DNA adducts [11], although other data found no adverse effect on DRC [12]. One study suggested the 194Trp variant has increased DRC [10]. Functional studies showed that the Arg280His variant has reduced cellular BER efficiency [13,14].

Epidemiological studies have shown significant associations between the Arg399Gln variant and various cancers (reviewed by Goode et al. [15]), but in bladder cancer the results have been inconsistent [16-18]. A recent meta-analysis of 38 case-control studies by Hu et al. [19] concluded that the Arg194Trp variant had a protective effect on cancer risk, while individuals carrying the Arg280His variant allele had increased cancer risk compared to those with the wildtype genotypes (odds ratio [95% confidence intervals], 1.19 [1.00–1.42]).

In addition to coding SNPs, non-coding XRCC1 polymorphisms may also affect DRC by altering the splice site or transcription efficiency. Recently, Hao et al. [20] discovered a novel T-77C polymorphism (rs3213245) in the XRCC1 gene which contributes to diminished promoter activity and increased risk of non-small cell lung cancer.

In the present study, we performed a comprehensive analysis of 14 potentially functional polymorphisms (coding and non coding) in XRCC1 to investigate their associations with bladder cancer. Furthermore, we constructed XRCC1 haplotypes and assessed interactions with smoking and occupational exposure.

Results

Subject characteristics

Demographic details for each subject have been described previously [21]. The majority of subjects were Caucasian (98.6%) with no difference in mean age (cases 72.8 years; controls 71.9 years). There was no significant difference in mean age of community and hospital controls (p = 0.19) and no difference in smoking or occupational exposure rates. There were however more men in the hospital group (P < 0.001) because of an attempt to obtain a similar overall sex ratio to the cases. Cases were more likely than controls to be smokers (78% vs. 64%, p < 0.001), male (70.9% vs. 65.5%, p = 0.05), have previous occupational exposure (27.4% vs. 16.8%, p < 0.001) and a positive family history of bladder cancer (4.8% vs. 2.2%, p = 0.02).

Haplotype analysis

We selected the three coding polymorphisms with amino acid substitutions previously commonly investigated, namely Arg194Trp, Arg280His and Arg399Gln to construct XRCC1 haplotypes. Four common haplotypes were estimated to account for over 99% of all haplotypes.
Recently, Hao et al. [20] in 1024 Chinese lung cancer patients and 1118 controls, showed that a 5'UTR SNP (T-77C, rs3213245), is associated with an increased risk of lung cancer, with OR for carriage of the variant allele of 1.46 (95% CI 1.18–1.82) compared to wildtype genotype. In functional studies the C allele-containing promoter showed no association for this SNP and increased bladder cancer risk.

Table 1: The linkage disequilibrium (LD) between all 14 XRCC1 polymorphisms

| No | Polymorphisms | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|----|---------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| 1  | EX1-1139D'    | 0.20 (0.13) | | | | | | | | | | | | |
| 2  | EX1-1128      | 0.82 (0.22) | | | | | | | | | | | | |
| 3  | EX1-900       | 0.92 (0.41) | | | | | | | | | | | | |
| 4  | EX-128        | 0.89 (0.95) | | | | | | | | | | | | |
| 5  | EX-52         | 0.90 (0.20) | | | | | | | | | | | | |
| 6  | Val72Al       | N/A | | | | | | | | | | | | |
| 7  | Arg194Trp     | 0.89 (0.97) | | | | | | | | | | | | |
| 8  | IVS7-33       | 0.04 (0.06) | | | | | | | | | | | | |
| 9  | Pro206        | 0.95 (0.39) | | | | | | | | | | | | |
| 10 | Arg280His     | 0.65 (0.74) | | | | | | | | | | | | |
| 11 | Arg399Gln     | 0.97 (0.97) | | | | | | | | | | | | |
| 12 | Gin632        | 0.86 (0.91) | | | | | | | | | | | | |
| 13 | EX17-127      | 0.89 (0.74) | | | | | | | | | | | | |

Values in bold italics represent the observed followed by estimated (in round bracket) control minor allele frequencies from public databases.

* LD is presented in pairwise D' followed by r² in round bracket.

N/A = Not available because of no pairwise variant genotypes in rare polymorphisms.

Values in bold are polymorphisms in high LD (D' ≥ 0.90 and r² ≥ 0.80).

Discussion

To date, this is the most comprehensive investigation on the association between XRCC1 polymorphisms and bladder TCC risk. We chose to study one gene comprehensively rather than studying a small number of SNPs in multiple genes, as others have done, for a number of reasons. Firstly, non-coding SNPs in the promoter region, 3' or 5'UTR or putative splice sites have been shown to influence DNA repair capacity [20]. Secondly this approach allows for construction and analysis of haplotypes and provides data on linkage disequilibrium between SNPs in the gene. We found none of the 14 XRCC1 polymorphisms was associated with bladder cancer risk. Our results on the Arg399Gln were consistent with other studies in bladder cancer [17,18]. Although our study was larger and had sufficient power, our results did not support the finding of Kelsey et al. [16] that the variant 399Gln was protective against bladder cancer. We also found no association between Arg194Trp and bladder cancer risk which is consistent with Wu et al. [18].

Recently, Hao et al. [20] in 1024 Chinese lung cancer patients and 1118 controls, showed that a 5'UTR SNP (T-77C, rs3213245), is associated with an increased risk of lung cancer, with OR for carriage of the variant allele of 1.46 (95% CI 1.18–1.82) compared to wildtype genotype. In functional studies the C allele-containing promoter showed no association for this SNP and increased bladder cancer risk.

Our data provided borderline evidence of association between Arg280His and bladder TCC with an estimated 50% increase in risk to individuals carrying the 280His variant (adjusted OR [95%CI], 1.50 [0.98–2.28]) but this study only had limited power (55%) to detect association with this variant. The association between XRCC1 Arg280His and an increased risk of bladder cancer is biologically plausible [13,14]. Takanami et al. [14] showed that the XRCC1 Arg280His variant protein is defective in its efficient localization to a damaged DNA site and thus impaired the cellular BER efficiency. Furthermore, the recent meta-analysis by Hu et al. [19] also found the 280His variant to be associated with increased cancer risk. However, the only previous bladder cancer study by Stern et al. [22] (235 cases and 213 controls) found no association. Our discrepant findings may be explained by false positive or false negative results, or the different populations studied.
Table 2: The association between the 14 XRCC1 polymorphisms and bladder cancer risk

| Polymorphisms* | Genotypes | Controls, n (%) | Cases, n (%) | aOR (95% CI) | P value |
|----------------|-----------|----------------|-------------|--------------|--------|
| EXI-1139       | C/C       | 328 (61.5)     | 295 (59.0)  | 1.00         | reference |
|                | rs2682586 | C/T            | 187 (35.1)  | 181 (36.2)   | 1.07 (0.82–1.39) | 0.62 |
| Promoter       | T/T       | 18 (3.4)       | 24 (4.8)    | 1.42 (0.74–2.71) | 0.29 |
| T allele freq. |           | 0.209          | 0.229       |              |        |
| EXI-1128       | rs2682585 | C/C C/T        | 319 (60.3)  | 189 (35.7)  | 287 (59.1) 177 (36.4) | 1.00 1.05 (0.81–1.37) | reference 0.71 |
| Promoter       | T/T       | 21 (4.0)       | 22 (4.5)    | 1.15 (0.61–2.18) | 0.66 |
| T allele freq. |           | 0.218          | 0.227       |              |        |
| EXI-900        |            | -/-            | 196 (35.5)  | 173 (34.0)  | 1.00         | reference |
|                | rs3213239 | +/-            | 267 (48.2)  | 256 (50.3)  | 1.04 (0.79–1.36) | 0.79 |
| Promoter       | +/-       | 91 (16.4)      | 80 (15.7)   | 1.02 (0.71–1.48) | 0.91 |
| + allele freq. |           | 0.405          | 0.409       |              |        |
| EX-128         |            | C/C            | 187 (33.6)  | 174 (32.8)  | 1.00         | reference |
|                | rs3213245 | C/T            | 275 (49.4)  | 266 (50.2)  | 1.00 (0.76–1.31) | 0.97 |
| 5'UTR          | T/T       | 94 (16.9)      | 90 (17.0)   | 1.06 (0.74–1.52) | 0.76 |
| T allele freq. |           | 0.416          | 0.421       |              |        |
| EX-52          |            | C/C            | 548 (97.2)  | 520 (97.6)  | 1.00         | reference |
|                | rs2307187 | C/T            | 16 (2.8)    | 13 (2.4)    | 0.91 (0.43–1.92) | 0.80 |
| 5'UTR          | T/T       | 0              | 0           | NC          | NC          |
| T allele freq. |           | 0.014          | 0.012       |              |        |
| Val72Ala       |            | C/C            | 562 (100)   | 521 (100)   | 1.00         | reference |
|                | rs25496   | C/T            | 0           | 0           | NC          | NC          |
| Exon 3         | T/T       | 0              | 0           | NC          | NC          |
| T allele freq. |           | 0              | 0           |              |        |
| Arg194Trp      |            | C/C            | 498 (88.6)  | 476 (89.0)  | 1.00         | reference |
|                | rs1799782 | C/T            | 61 (10.9)   | 56 (10.4)   | 0.95 (0.64–1.41) | 0.81 |
| Exon 6         | T/T       | 3 (0.5)        | 3 (0.6)     | 1.01 (0.19–5.23) | 0.99 |
| C/T + T/T      |          | 64 (11.4)      | 59 (11.0)   | 0.95 (0.65–1.40) | 0.81 |
| T allele freq. |           | 0.060          | 0.058       |              |        |
| IVS7-33        |            | C/C            | 518 (91.7)  | 484 (89.6)  | 1.00         | reference |
| Polymorphism   | Reference | n   | |
|---------------|-----------|-----|-------|
| rs1799780     | C/T      | 47  | (8.3) |
| Intronic      | T/T      | 0   | (0)   |
|               | C/T + T/T| 47  | (8.3) |
| T allele freq.|           | 0.042|       |
| Pro206Pro     | G/G      | 170 | (31.2) |
| rs915927      | G/A      | 270 | (49.5) |
| Exon 7        | A/A      | 105 | (19.3) |
| A allele freq.|           | 0.440|       |
| Arg280His     | G/G      | 516 | (92.1) |
| rs25489       | G/A      | 41  | (7.3)  |
| Exon 9        | A/A      | 3   | (0.6)  |
| G/A + A/A     | 44       | (7.9)|       |
| A allele freq.|           | 0.042|       |
| Arg399Gln     | G/G      | 226 | (40.4) |
| rs25487       | G/A      | 259 | (46.2) |
| Exon 10       | A/A      | 75  | (13.4) |
| A allele freq.|           | 0.365|       |
| Gln632Gln     | G/G      | 176 | (31.4) |
| rs35474       | G/A      | 275 | (49.0) |
| Exon 17       | A/A      | 110 | (19.6) |
| A allele freq.|           | 0.441|       |
| EX17-123      | /-       | 492 | (89.5) |
| rs3213401     | /+       | 55  | (10.0) |
| 3'UTR         | +/+      | 3   | (0.5)  |
|               | /+ plus +/+| 58 | (10.5)|       |
| + allele freq.|           | 0.055|       |
| EX17-127      | G/G      | 337 | (64.1) |
| rs2682558     | G/A      | 162 | (30.8) |
| 3'UTR         | A/A      | 27  | (5.1)  |
| A allele freq.|           | 0.205|       |

* Polymorphisms included name, dbSNP reference number and its location in XRCC1 gene.
aOR = odds ratio adjusted for subject’s age, gender, smoking, occupational exposure and family history
- = wildtype without insertion, + = variant with insertion of GGGAATC
This study was potentially limited by selection bias since both community and hospital controls were included. Although there were differences in the genotype distributions of one polymorphism significant at the 5% level, we believe it unlikely that use of these two control groups influenced the results. Recall bias for smoking, occupational exposure and family history cannot be excluded. Our results must be interpreted cautiously bearing in mind multiple testing as 14 XRCC1 polymorphisms were selected. However, these tests are highly correlated since numbers of polymorphisms were in almost perfect LD.

Exposure to tobacco smoke or to the occupational risk factors studied causes damage to DNA, and these exposures have been shown to increase the risk of bladder cancer. Exposure to these chemicals in an individual with an inefficient DNA repair mechanism might be expected to elevate risk more substantially. We therefore tested the hypothesis that the joint effect on risk of exposure and genotype was greater than multiplicative (gene-environment interaction). Stratified analysis by smoking and chemical exposure did not show any significant gene environment interaction. Interestingly, the 280His variant allele seemed to have a greater influence on bladder cancer risk particularly among current smokers (adjusted OR [95%CI], 2.51 [0.87–7.31], p = 0.09) but the likelihood ratio test for interaction was not significant (p = 0.45). A few other studies have investigated interactions between XRCC1 polymorphisms and smoking on bladder cancer risk [16,23,24]. None has shown evidence of interaction, although it should be borne in mind that most studies to date, including our own, only have sufficient power to detect strong interactions.

Conclusion
This study provides no evidence of an association between polymorphisms in XRCC1 and bladder cancer risk. However, although the study is relatively large (547 cases and 579 controls), there is limited power to detect the association for low frequency variants such as Arg280His, and the data are consistent with a modestly increased risk for carriers of the variant allele.

Methods
Study population
A detailed description of the study population and design has been given elsewhere [21]. In brief, 547 cases of bladder transitional cell carcinoma (TCC) were recruited from August 2002 to April 2004 at our institution in Leeds, UK, after obtaining ethical approval from the Leeds (East) Local Research Ethical Committee. Cancer-free controls (n = 579) were recruited from the community from 1997 to 2000 (n = 227) as part of a previous colorectal cancer study [25] and from the hospital otolaryngology and oph-

### Table 3: Stratified analysis by smoking and occupational status for XRCC1 Arg280His polymorphism

| Exposure status | Genotypes | Control (n) | Cases (n) | Crude OR (95%CI) | Adjusted OR (95%CI) | P value |
|-----------------|-----------|-------------|-----------|------------------|---------------------|---------|
| Smoking         | G/G       | 174         | 103       | 1.00             | 1.00                | 0.77    |
| Non-smoker      | G/A + A/A | 16          | 12        | 1.27 (0.58–2.78) | 1.13 (0.50–2.56)    | 0.38    |
| Ex smoker       | G/G       | 253         | 244       | 1.00             | 1.00                | 0.09    |
| Current smoker  | G/G       | 89          | 108       | 1.00             | 1.00                |         |
|                 | G/A + A/A | 5           | 16        | 2.64 (0.93–7.48) | 2.52 (0.87–7.31)    |         |

| Occupation      | Genotypes | Control (n) | Cases (n) | Crude OR (95%CI) | Adjusted OR (95%CI) | P value |
|-----------------|-----------|-------------|-----------|------------------|---------------------|---------|
| No occupational | G/G       | 429         | 327       | 1.00             | 1.00                | 0.07    |
| exp             | G/A + A/A | 35          | 43        | 1.61 (1.01–2.58) | 1.55 (0.96–2.51)    |         |
| Occupational exp| G/G       | 87          | 129       | 1.00             | 1.00                |         |
|                 | G/A + A/A | 9           | 14        | 1.05 (0.43–2.53) | 1.03 (0.42–2.54)    |         |

* Adjusted OR for subject’s age, gender, smoking, occupational exposure and family history

### Table 4: Estimated XRCC1 haplotype frequencies in cases and controls

| Arg194Trp | Arg280His | Arg399Gln | Controls haplotype (%) | Cases haplotype (%) |
|-----------|-----------|-----------|------------------------|---------------------|
| C         | G         | G         | 52.9                   | 52.6                |
| C         | G         | A         | 36.9                   | 36.1                |
| C         | A         | G         | 4.2                    | 5.5                 |
| T         | G         | G         | 6.0                    | 5.8                 |

| Total     | 100.0     | 100.0     |

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thalamology departments in 2002 to 2004 (n = 352) in the same region. The participation rate for cases and controls were 99% and 80% respectively.

Each subject donated a blood sample and completed a structured health questionnaire regarding smoking, occupational and family history. Occupational exposure was defined as participating in occupations involving rubber/plastics industries, laboratories, printing, paints, dyes or diesel fumes.

**Genotyping**

We selected XRCC1 polymorphisms based on potential function from the public domain of the Environmental Genome Project (EGP) via the National Centre for Biotechnology Information[26]. XRCC1 polymorphisms with allele frequencies of more than 1% located in the promoter region (up to 1000 bases upstream of the gene), 5’ and 3’ untranslated regions (UTR), exons, and intronic regions with known or potential splicing effects (within 100 bases up and downstream of exons) were selected (Table 1).

Genotyping was performed using the allelic discrimination 5’ nuclease assay (Taqman) without prior knowledge of the subject’s clinical status, as previously described [21]. Primers and probes are listed in Table 5. As a quality control measure, 5% of the samples (n = 57) were regenotyped with 100% concordance.

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**Statistical analysis**

All data were analyzed using Stata Statistical Software Release 8 (Stata Corporation, 2003, College Station, Texas). Hardy-Weinberg equilibrium analyses were performed to compare observed and expected genotype frequencies using a chi-squared goodness-of-fit test. The linkage disequilibrium (LD) between polymorphisms was estimated by pairwise Lewontin’s D’ and r² using the STATA pwld function [27]. The odds ratio (OR) and 95% confidence interval (CI) for bladder cancer associated with each genotype were calculated using logistic regression analysis, both without adjustment and adjusted for established bladder cancer risk factors (age, sex, cigarette smoking, occupational exposure and family history).

Haplotypes were estimated by the Expectation Maximization (EM) algorithm [28] and their frequencies in cases and controls compared using a likelihood ratio test with a permutation procedure to obtain empirical p-values as implemented in EHPLUS [29]. With a sample size of 547 cases and 579 controls, we had an 90%, 78% and 55% power to detect an OR of 1.50 for carriage of the minor allele of frequency 0.20, 0.10 and 0.05 respectively (2 sided, p = 0.05). Gene-environment interaction was assessed by stratification of subjects based on smoking status and occupational exposure, and p values are calculated based on the likelihood-ratio test, comparing models with and without an interaction term.

**Authors’ contributions**

SCS recruited the cases and the majority of the controls, co-ordinated the genotyping, performed the main statistical analyses and interpretation of results, drafted the manuscript.

JHB participated in the design of the study, advised on and carried out some of the statistical analyses and interpreted the results, and helped to draft and redraft the manuscript.

ABP gave clinical advice on patients, participated in the design of the study and critically appraised the manuscript.

DTB helped in the conception of the study, the interpretation of the results and redrafting of the manuscript.

Table 5: List of primers and probes for 14 XRCC1 polymorphisms

| Polymorphisms | Forward primers | Reverse primers | VIC probes | FAM probes |
|---------------|-----------------|-----------------|------------|------------|
| rs2682558     | GTCCCAGATTGAGAAGAGAGAT | CCCGTTACCTGTGAGGCCTG | CTTAGGCGCTCTCTCT | TGAAGGCTCTTCTCT |
| rs2682558     | CTCCCCCGTAGGGTAAGTG | CTGTCGCCCACTTTTTATAGGAA | CAGACCACCGCCCTC | CAGACCCACCCCTC |
| rs3213235     | CCCGTTTTGCGACCTATTAG | CAGACCACCGCCCTC | CAATGGGCGCCTGGT | CAAACAACATGGGCCGT |
| rs2307187     | CCCCATATCTACCTCATCCTTCTTCTTG | GCTGCAGGATGATGTTGGAAGTGCAG | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |
| rs2307187     | CTGCAGGAGGACCGCCCT | CTGGTCCCCAGCTTTTATAGGAAGT | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |
| rs1997982     | AAGTAGAGGAGGACCT | AACTGCTGCTGTACCC | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |
| rs1997898     | CCATAGATGAGGGAGAGGT | GCTCATGCTGCTTGATGAGATGG | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |
| rs195927      | TCCACCTTCTCCCTCTATGCAGC | AGGGTAGAAGCTCTCTAGGAGC | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |
| rs32489       | CCAGTGGTGCTACTAACCATACTACTC | GCTGCGAGCGGGACTG | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |
| rs32487       | GTGGTGCTGGACTGTC | GCAGGCGTGGCGGTGGTG | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |
| rs3123401     | GAGTGTGTCCTCTATCCAGAAG | AAGATAGGGTGGTGTCGTCCT | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |
| rs3662558     | CCTATCCCTGTTGTGGGCAAGAAG | GCCTAGAGGGAGGGCAAGAA | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |
| rs3547        | TGTTGTGTTGTGTGTATAGGCA | GCAGAAGTACTCTCCACTACCTCTC | CAGAGGCTCTCTCTCT | TGAAGGCTCTTCTCT |

Probes are not required because the variant allele involved deletion of 7 nucleotides which is sufficient to be detected by PCR product size.
AEK conceived the study, facilitated the genotyping and helped to redraft the manuscript. All authors read and approved the final manuscript.

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References
1. Boyle P, Ferlay J: Cancer incidence and mortality in Europe, 2004. Ann Oncol 2005, 16(3):481-488.
2. Kogevinas M, t Mannetje A, Cordier S, Ranft U, Gonzalez CA, Vineis P, Chang-Claude J, Lynge E, Wahrendorf J, Tzonou A, jocket KH, Surr C, Parru S, Hours M, Greire E, Boffetta P: Occupation and bladder cancer among men in Western Europe. Cancer Causes Control 2003, 14(10):907-914.
3. Zeegers MP, Tan FE, Dorant E, van Den Brandt PA: DNA repair gene XRCC1 modulates the genotoxic response risk: a meta-analysis of epidemiologic studies. Cancer 2000, 89(3):630-639.
4. Berwick M, Vineis P: Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. J Natl Cancer Inst 2000, 92(11):874-897.
5. Mohrenweiser HW, Jones IM: 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 1998, 400(1-2):15-24.
6. Caldecott KW: XRCC1 and DNA strand break repair. DNA Repair (Amst) 2003, 2(9):955-969.
7. Tebbs RS, Flannery ML, Meneses JJ, Hartmann A, Tucker JD, Thompson LH, Cleaver JE, Pedersen RA: Requirement for the Xrccl1 DNA base excision repair gene during early mouse development. Dev Biol 1999, 208(2):513-529.
8. Thompson LH, West MG: XRCC1 keeps DNA from getting stranded. Mutat Res 2000, 459(1):1-18.
9. Abdel-Rahman SZ, El-Zaín RA: The 399Gln polymorphism in the XRCC1 gene modulates the genotoxic response induced in human lymphocytes by the tobacco-specific nitrosamine NNK. Cancer Lett 2000, 159(1):63-71.
10. Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA: XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycolphosphory A variant frequency. Cancer Res 1999, 59(11):2557-2561.
11. Duell EJ, Wiencke JK, Cheng Tj, Varkonyi A, Zuo ZF, Ashok TD, Mark EJ, Wain JC, Christiani DC, Kelsey KT: Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis 2000, 21(5):965-971.
12. Taylor RM, Thistlethwaite A, Caldecott KW: Central role for the XRCC1BRCT 1 domain in mammalian DNA single-strand break repair. Mol Cell Biol 2002, 22(8):2556-2563.
13. Pachkowski BF, Winkiel S, Kubota Y, Swenborg JA, Millican RC, Nakamura J: XRCC1 genotype and breast cancer: functional studies and epidemiologic data show interactions between XRCC1 codon 280 His and smoking. Cancer Res 2006, 66(5):2860-2868.
14. Takanami T, Nakamura J, Kubota Y, Horiechi S: The Arg280His polymorphism in X-ray repair cross-complementing gene 1 impairs DNA repair ability. Mutat Res 2005, 582(1-2):135-145.
15. Goode EL, Ulrich CM, Potter JD: Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002, 11(12):1513-1530.
16. Kelsey KT, Park S, Nelson HH, Karagas MR: A population-based case-control study of the XRCC1 Arg399Gln polymorphism and susceptibility to bladder cancer. Cancer Epidemiol Biomarkers Prev 2004, 13(8):1337-1341.
17. Sanyal S, Festa F, Sakano S, Zhang Z, Steineck G, Norming U, Wikstrom H, Larsson P, Kumar R, Hemminki K: Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 2004, 25(5):729-734.
18. Wu X, Gu J, Grossman HB, Amos CI, Ezel C, Huang M, Zhang Q, Miinoge RE, Lerner S, Dinney CP, Spitz MR: Bladder Cancer Pre-disposition: A Multigene Approach to DNA-Repair and Cell-Cycle-Control Genes. Am J Hum Genet 2006, 78(3):464-479.
19. Hu Z, Ma H, Chen F, Wei Q, Shen H: XRCC1 polymorphisms and cancer risk: a meta-analysis of 38 case-control studies. Cancer Epidemiol Biomarkers Prev 2005, 14(7):1810-1818.
20. Hao B, Wang H, Zhou K, Li Y, Chen X, Zhou G, Zhu Y, Miao X, Tan W, Wei Q, Lin D, He F: Identification of genetic variants in base excision repair pathway and their associations with risk of esophageal squamous cell carcinoma. Cancer Res 2004, 64(12):4378-4384.
21. Sak SC, Barrett JH, Paul AB, Bishop DT, Kiltie AE: The polyAT, intronic IVS11-6 and Lys939Gln XPC polymorphisms are not associated with transitional cell carcinoma of the bladder. Br J Cancer 2005, 92(12):2262-2265.
22. Sierro MC, Umbach DM, van Gils CH, Lunn RM, Taylor JA: DNA Repair Gene XRCC1 Polymorphisms, Smoking, and Bladder Cancer Risk. Cancer Epidemiol Biomarkers Prev 2001, 10(2):125-131.
23. Matullo G, Guarrera S, Sacerdote C, Polidoro L, Davico L, Gambineri S, Karagas M, Cassetta G, Rolle L, Piazza A, Vineis P: Polymorphisms/haplotypes in DNA repair genes and smoking: a bladder cancer case-control study. Cancer Epidemiol Biomarkers Prev 2005, 14(1Pt1):2569-2578.
24. Shen M, Hung RJ, Brennan P, Malaveille C, Donato F, Placidi D, Carta A, Hueteauille A, Boffetta P, Perro S: Polymorphisms of the DNA repair genes XRCC1, XRCC3, XPD, interaction with environmental exposures, and bladder cancer risk in a case-control study in northern Italy. Cancer Epidemiol Biomarkers Prev 2003, 12(11 Pt1):1234-1240.
25. Barrett JH, Smith G, Waxman R, Godderham N, Lightfoot T, Garner RC, Augustsson K, Wolf CR, Bishop DT, Forman D: Investigation of interaction between N-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer. Carcinogenesis 2003, 24(2):275-282.
26. The Environmental Genome Project SNPs Database for XRCC1 gene [http://www.ncbi.nlm.nih.gov/SNP/snp_viewBatch.cgi? bid=5410]
27. Clayton D: David Clayton’s programs for genetic analysis. [http://www.gene.cimr.cam.ac.uk/clayton/software/]
28. Excoffier L, Slatkin M: Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 1995, 12:921-927.
29. Zhao JH, Curtis D, Sham PC: Model-free analysis and permutation tests for allelic associations. Hum Hered 2000, 50(2):133-139.