Original Article
Current evidence on XPC rs2228001 A/C polymorphism and bladder cancer susceptibility

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Abstract: The xeroderma pigmentosum group C (XPC) gene plays a significant role in DNA damage recognition during nucleotide excision repair process. Polymorphisms of the XPC gene have been analyzed in numerous case-control studies to evaluate bladder cancer risk attributed to XPC genetic variation; however, published data on the association between XPC rs2228001 A/C and bladder cancer risk are inconclusive. To assess the impact of XPC rs2228001 polymorphism on bladder cancer risk, we performed a meta-analysis on all available studies including 4,741 patients and 5,065 control subjects. The overall results indicated a positive association of the variant on bladder cancer risk using an allelic contrast, homozygote comparison and the recessive genetic model. When stratified by ethnicity, obvious associations were found in Asian descendants by using an allelic contrast (odds ratio [OR] = 1.33, 95% confidence interval [CI] = 1.06-1.66, P heterogeneity = 0.002), homozygote comparison (OR = 1.91, 95% CI = 1.30-2.82, P heterogeneity = 0.040) and the recessive genetic model (OR = 1.64, 95% CI = 1.25-2.15, P heterogeneity = 0.128). Furthermore, a significant association was found in smokers by using an allelic contrast (OR = 2.08, 95% CI = 1.04-4.17, P heterogeneity = 0.039), homozygote comparison (OR = 4.66, 95% CI = 1.22-17.76, P heterogeneity = 0.024) and the recessive genetic model (OR = 3.79, 95% CI = 1.18-12.20, P heterogeneity = 0.027). In conclusion, XPC rs2228001 A/C polymorphism may contribute to the risk for developing bladder cancer in Asian descendants and may interact with smoking exposure. Further studies based on larger, more diverse case-control populations are warranted to further evaluate the association.

Keywords: XPC, polymorphism, bladder cancer, meta-analysis

Introduction

Transitional cell carcinoma of the bladder is one of the most common types of neoplasm in the Western world [1]. In the United States, bladder cancer is the fifth most prevalent cancer, with 70,980 new cases predicted to occur in 2009 [2]. In China, bladder cancer was the tenth most common cancer in 2005 [3]. Epidemiological studies indicate that cigarette smoking is strongly associated with the bladder carcinoma risk. Cigarette smoke contains several potent chemical carcinogens that are estimated to contribute up to approximately 65% of bladder cancer occurrence in men and up to 20-30% in women [4, 5]. Carcinogens from tobacco smoking cause DNA damage via the introduction of crosslinks, bulky adducts, and single or double-stranded breaks [6].

Repairing damaged DNA is the prevalent defense mechanism against mutagenic exposure. Many chemical carcinogenic metabolites generate bulky DNA adducts, which are mainly removed by the nucleotide excision repair (NER) pathway. This pathway also repairs endogenously generated oxidative DNA lesions [7]. Xeroderma pigmentosum group C (XPC), spans 33 kb on chromosome 3 and includes 16 exons and 15 introns. XPC encodes a protein that recognizes and binds to helix-distorting DNA adducts and plays a significant role in damage detection and initiation of the NER pathway [8, 9]. XPC mutations can lead to xeroderma pig-
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mentosum, a rare autosomal recessive disease with accumulated sensitivity to sunlight and the development of skin carcinoma at an early age [10]. XPC knockout mice demonstrate a defective NER pathway, developing skin carcinomas following exposure to ultraviolet B radiation and have increased susceptibility to common cancers, including bladder, lung and liver cancer, following exposure to the chemical carcinogen, acetylaminofluorene [11, 12].

Numerous epidemiological studies have been conducted to explore the association of XPC polymorphisms with bladder cancer risk. One of the most widely investigated XPC polymorphism is an A to C substitution in exon 15 (rs-2228001) at position 939 (Lys939Gln), resulting in a lysine-to-glutamine substitution. However, the association between XPC rs2228001 A/C polymorphism and bladder cancer risk remains controversial due to conflicting results from various case-control studies. Hence, we utilized the enhanced statistical power of a meta-analysis to obtain a summary result on the association of XPC rs2228001 A/C polymorphism bladder cancer using accumulated data from eligible studies published to date [13-24]. Additionally, the potential interaction between XPC rs2228001 A/C polymorphism and total smoking exposure on bladder cancer risk was evaluated.

Materials and methods

Search strategy and identification of relevant studies

PubMed database searches were conducted using the following keywords: ‘Xeroderma pigmentosum group C’ or ‘XPC’, ‘bladder cancer’ and ‘polymorphism’ (last search updated on August 01, 2015). References of the identified manuscripts were also manually screened for eligibility. Eligible manuscripts met all of the following criteria: (a) used a case-control design with unrelated individuals; (b) contained information about genotype frequency; (c) the genotype distribution of the control population was in accord with Hardy-Weinberg equilibrium (HWE); (d) the study was published in English; and (e) was a full-text article. The following exclusion criteria were utilized: (1) study lack of available genotype frequency data; (2) lack of the control population; (3) study was the duplicate.

Data extraction and quality assessment

For each eligible publication, data extraction and methodological quality assessment were conducted by two of the co-authors independently. Disagreement was resolved by discussion between the two co-authors. If a consensus could not be reached, additional co-authors were included in the discussion until a consensus was reached. The following parameters from each study were recorded: first author’s name, publication date, ethnicity, the source of cancer cases and controls, sample size of cases and controls, and the number of cases and controls with wild type and variant allele, respectively.

Statistical analysis

Crude odds ratios (ORs) with 95% confidence intervals (CIs) were utilized to evaluate the strength of the association between XPC rs-2228001 polymorphism and bladder cancer based on the genotype frequencies in cases and controls. For XPC rs2228001 A/C, we investigated the relationship between genetic variants and bladder cancer risk in allelic contrast (C-allele vs. A-allele), homozygote comparison (CC vs. AA), heterozygote comparison (CA vs. AA), dominant genetic model (CC + CA vs. AA) and recessive genetic model (CC vs. CA + AA). Subgroup analyses were conducted, stratifying by ethnicity, source of control subject (hospital-based or population-based), and smoking exposure. We utilized the random effects model and fixed effects model to calculate the pooled OR. The heterogeneity assumption was evaluated by a chi-square-based Q-test among the studies. P values greater than 0.05 for the Q-test indicates lack of heterogeneity among studies, in which case the pooled OR was examined using a fixed-effects model (the Mantel-Haenszel method) [25]; otherwise, a random effects model (DerSimonian and Laird method [26]) was performed. Significant departures of allele frequencies of XPC rs2228001 polymorphism from expectation under HWE were evaluated in controls utilizing the Pearson’s chi-square test. P < 0.05 was considered statistically significant. A Z-test was used to evaluate the statistical significance of the summary OR with a P value of < 0.05 considered significant. We also utilized the statistic of I^2 to efficiently test for heterogeneity, with I^2 > 75%, 25-75% and < 25% to representing a high,
Table 1. Study characteristics of XPC rs2228001 A/C polymorphism included in this meta-analysis

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Source of control</th>
<th>Genotyping methods</th>
<th>Sample size of case</th>
<th>Sample size of control</th>
<th>P HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wen</td>
<td>2013</td>
<td>China</td>
<td>Asian</td>
<td>Hospital-based</td>
<td>TaqMan</td>
<td>25</td>
<td>56</td>
<td>49</td>
</tr>
<tr>
<td>Zhi</td>
<td>2012</td>
<td>China</td>
<td>Asian</td>
<td>Hospital-based</td>
<td>PCR-RFLP</td>
<td>48</td>
<td>136</td>
<td>118</td>
</tr>
<tr>
<td>Mittal</td>
<td>2012</td>
<td>India</td>
<td>Asian</td>
<td>Hospital-based</td>
<td>PCR-RFLP</td>
<td>28</td>
<td>73</td>
<td>94</td>
</tr>
<tr>
<td>Liu</td>
<td>2012</td>
<td>China</td>
<td>Asian</td>
<td>Hospital-based</td>
<td>PCR-RFLP</td>
<td>92</td>
<td>272</td>
<td>236</td>
</tr>
<tr>
<td>Rouissi</td>
<td>2011</td>
<td>Tunisia</td>
<td>African</td>
<td>Hospital-based</td>
<td>PCR-RFLP</td>
<td>20</td>
<td>52</td>
<td>53</td>
</tr>
<tr>
<td>Verdié</td>
<td>2010</td>
<td>Sweden</td>
<td>European</td>
<td>Population-based</td>
<td>PCR-RFLP</td>
<td>51</td>
<td>141</td>
<td>113</td>
</tr>
<tr>
<td>Gangwar</td>
<td>2010</td>
<td>India</td>
<td>Asian</td>
<td>Hospital-based</td>
<td>PCR-RFLP</td>
<td>25</td>
<td>86</td>
<td>97</td>
</tr>
<tr>
<td>Fontana</td>
<td>2008</td>
<td>France</td>
<td>European</td>
<td>Hospital-based</td>
<td>TaqMan</td>
<td>22</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Zhu</td>
<td>2007</td>
<td>USA</td>
<td>European</td>
<td>Population-based</td>
<td>TaqMan</td>
<td>80</td>
<td>271</td>
<td>199</td>
</tr>
<tr>
<td>García</td>
<td>2006</td>
<td>Spain</td>
<td>European</td>
<td>Population-based</td>
<td>SNP500Cancer</td>
<td>188</td>
<td>575</td>
<td>374</td>
</tr>
<tr>
<td>Wu</td>
<td>2006</td>
<td>USA</td>
<td>European</td>
<td>Population-based</td>
<td>PCR-RFLP</td>
<td>94</td>
<td>293</td>
<td>219</td>
</tr>
<tr>
<td>Sak</td>
<td>2005</td>
<td>UK</td>
<td>European</td>
<td>Population-based</td>
<td>PCR-RFLP</td>
<td>87</td>
<td>241</td>
<td>204</td>
</tr>
</tbody>
</table>

HWE: Hardy-Weinberg equilibrium of controls, RFLP: restriction fragment length polymorphism.

The meta-analysis was consistent with HWE in all the publications. Study characteristics of eligible publications are summarized in Table 1. The meta-analysis included 4,741 bladder cancer patients and 5,065 control subjects. Six of the studies were performed with European descendants, 5 with Asian descendants and one with African descendants. Hospital-based controls were used in 6 of the studies. Polymerase chain reaction-restriction fragment length polymorphism (RFLP), the classical genotyping method, was used in 7 comparisons while 4 studies utilized TaqMan real-time polymerase chain reaction (PCR). Furthermore, 4 publications included smoking status (categorized as smokers and non-smokers).

Results

Study characteristics

A total of 12 publications met all inclusion criteria and were included in the meta-analysis. The genotype distribution in the control population was consistent with HWE in all the publications. Study characteristics of eligible publications are summarized in Table 1. The meta-analysis included 4,741 bladder cancer patients and 5,065 control subjects. Six of the studies were performed with European descendants, 5 with Asian descendants and one with African descendants. Hospital-based controls were used in 6 of the studies. Polymerase chain reaction-restriction fragment length polymorphism (RFLP), the classical genotyping method, was used in 7 comparisons while 4 studies utilized TaqMan real-time polymerase chain reaction (PCR). Furthermore, 4 publications included smoking status (categorized as smokers and non-smokers).

Quantitative synthesis

Using the pooled data, (Table 2) a positive association of the XPC variant on bladder cancer risk was found using an allelic contrast (random-effects OR = 1.18, 95% CI = 1.04-1.33, P heterogeneity < 0.001, P = 0.009, I² = 74.2), homozygote comparison (random-effects OR = 1.44, 95% CI = 1.12-1.85, P heterogeneity < 0.001, P = 0.004, I² = 71.6) and the recessive genetic model (random-effects OR = 1.38, 95% CI = 1.10-1.72, P heterogeneity < 0.001, P = 0.005, I² = 69.2). Further, in a subgroup analysis stratified by ethnicity, obvious associations between XPC rs2228001 A/C polymorphism and bladder cancer risk were observed in Asian descendants using an allelic comparison (random-effects OR = 1.33, 95% CI = 1.06-1.66, P heterogeneity = 0.002, P = 0.015, I² = 76.2), but not in European descendants (fixed-effects OR = 1.07, 95% CI = 0.93-1.23, P heterogeneity = 0.006, P = 0.367, I² = 69.1) nor African descendants (OR = 1.15, 95% CI = 0.80-1.66, P = 0.454) (Figure 1). A significant dose-response association was found in smokers by using an allelic contrast (random-effects OR = 2.08, 95% CI = 1.04-4.17, P heterogeneity < 0.001, P = 0.039, I² = 86.8) (Figure 2), homozygote comparison (random-effects OR = 4.66, 95% CI = 1.22-17.76, P heterogeneity = 0.024, P = 0.013, I² = 72.3) and the recessive genetic model (random-effects OR = 3.79, 95% CI = 1.18-12.20, P heterogeneity = 0.027, P = 0.026, I² = 67.2). Furthermore, a significant association between the XPC rs2228001 polymorphism and bladder cancer was also found in hospital-based studies by using an allelic comparison (random-effects OR = 1.46, 95% CI = 1.13-1.89, P heterogeneity = 0.001, P = 0.030, I² = 74.8), homozygote comparison (random-effects OR = 2.26, 95% CI = 1.47-3.48, P heterogeneity = 0.045, P < 0.001, I² = 56.0), the dominant genetic model (random-effects OR = 1.40, 95%
## Table 2. Stratified analyses of the XPC rs2228001 A>C polymorphism on urinary-bladder cancer risk

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Cases/Controls</th>
<th>C-allele vs. A-allele</th>
<th>CC vs. AA</th>
<th>CC vs. CA+AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>I²</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>4741/5065</td>
<td>1.18 (1.04-1.33)</td>
<td>&lt; 0.001</td>
<td>0.009</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>6</td>
<td>3181/3222</td>
<td>1.07 (0.93-1.23)</td>
<td>0.006</td>
<td>0.367</td>
</tr>
<tr>
<td>Asian</td>
<td>5</td>
<td>1435/1718</td>
<td>1.33 (1.06-1.66)</td>
<td>0.002</td>
<td>0.015</td>
</tr>
<tr>
<td>African</td>
<td>1</td>
<td>125/125</td>
<td>1.15 (0.80-1.66)</td>
<td>-</td>
<td>0.454</td>
</tr>
<tr>
<td>Source of control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital-based</td>
<td>6</td>
<td>1011/1279</td>
<td>1.46 (1.13-1.89)</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>Population-based</td>
<td>6</td>
<td>3730/3786</td>
<td>1.02 (0.97-1.07)</td>
<td>0.269*</td>
<td>0.446</td>
</tr>
<tr>
<td>Smoking exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>4</td>
<td>386/693</td>
<td>1.15 (0.92-1.44)</td>
<td>0.213</td>
<td>0.070</td>
</tr>
<tr>
<td>Smokers</td>
<td>4</td>
<td>503/389</td>
<td>2.08 (1.04-4.17)</td>
<td>0.039</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Number of comparisons, *P* value of *Q*-test for heterogeneity test (*P* _het_). Random effects model was performed when *P* _het_ < 0.05; otherwise, fixed effects model was used.
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Figure 1. Forest plot of bladder cancer risk associated with the XPC rs2228001 A/C polymorphism (allelic contrast of C-allele vs. A-allele) in the stratified analyses by ethnicity. The squares and horizontal lines represent the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond corresponds to the summary OR and 95% CI. Separate details were summarized in Table 1.

Figure 2. Association between the XPC rs2228001 A/C polymorphism, smoking and the risk of bladder cancer, evaluated by the allelic contrast (C-allele vs. A-allele). The area of the squares reflects the weight. The squares and horizontal lines represent the study-specific OR and 95% CI. The diamond corresponds to the summary OR and 95% CI.

CI = 1.01-1.94, \( P_{\text{heterogeneity}} = 0.006, P = 0.043, I^2 = 69.5 \), and the recessive genetic model (random-effects OR = 1.83, 95% CI = 1.40-2.40, \( P_{\text{heterogeneity}} = 0.235, P < 0.001, I^2 = 26.6 \)), while no association existed among population-based studies (allelic comparison: fixed-effects OR = 1.02, 95% CI = 0.97-1.07, \( P_{\text{heterogeneity}} = 0.269, P = 0.446, I^2 = 21.9 \); homozygote comparison: fixed-effects OR = 1.05, 95% CI = 0.93-1.19, \( P_{\text{heterogeneity}} = 0.160, P = 0.444, I^2 = 37.0 \); dominant genetic model: fixed-effects OR = 1.01, 95% CI = 0.98-1.05, \( P_{\text{heterogeneity}} = 0.358, P = 0.492, I^2 = 9.1 \); recessive genetic model: fixed-effects OR = 1.06, 95% CI = 0.91-1.22, \( P_{\text{heterogeneity}} = 0.103, P = 0.478, I^2 = 45.4 \).

Publication bias

The Egger’s test and Begg’s funnel plot were carried out to assess potential publication bias in the literature. The shape of the funnel plots seemed asymmetrical in allelic comparison for XPC rs2228001 A/C polymorphism, suggesting no publication bias (Figure 3). Egger’s test was also conducted to evaluate the publication bias to provide statistical evidence of funnel plot symmetry, and data did not reveal evidence of publication bias (Table 3).

Discussion

Genetic susceptibility to cancers has led to increased attention to polymorphisms in genes involved in the process of carcinogenesis. Accumulating evidence demonstrates that reduced DNA repair capacity due to genetic variation is associated with an increased risk for human malignancies [29, 30]. Among them, bladder cancer is strongly associated with environmental and occupational exposure to chemical carcinogens. Given the high prevalence of cigarette smoking, it is likely responsible for more cases of bladder cancer than any other
risk factor [31]. The damage from cigarette smoke is predominantly repaired by the NER pathway that includes XPC [32]. Nevertheless, the association between XPC rs2228001 A/C polymorphism and bladder cancer risk remains inconclusive. For example, de Verdier et al. demonstrated that the XPC rs2228001 A/C variant significantly increased cancer risk [15]. However, Liu et al. did not find this variation to be associated with an elevated bladder cancer risk [13]. The goal of this meta-analysis was to combine results from previous research to yield summary conclusions, which is quite useful when individual studies may have been too small to achieve a valid conclusion. In addition, our meta-analysis revealed new information regarding the association of XPC rs2228001 polymorphism with bladder cancer.

Our results suggest that XPC rs2228001 A/C polymorphism is associated with increased bladder cancer risk in Asian descendants (under allelic contrast, homozygote comparison and the recessive genetic model) in a stratified analysis. Furthermore, a significant association was observed in smokers using an allelic contrast, homozygote comparison and the recessive genetic model when stratified by smoking exposure. In the analysis stratified by the source of control subjects, the XPC rs2228001 variant was found to increase bladder cancer risk in hospital-based studies (using an allelic contrast, homozygote comparison, and the dominant and recessive genetic models). However, some challenges may limit the generalization of these results. First, the diversity of ethnic backgrounds was limited with only one study using African descendants while 5 were based on Asian descendants. In addition, only four publications included information on the interaction of smoking exposure with XPC rs2228001 polymorphism on bladder cancer risk. Second, the studies may have differed in the histological types of carcinoma that may be associated with different susceptibility. Although we attempted to evaluate the effect of XPC rs2228001 polymorphism on the susceptibility in different types of bladder cancer, the available data were too limited. Third, positive results tend to be published more quickly than 'negative' results, creating a time-lag bias [33]. Fourth, other potential environmental-gene interactions including dietary factors, radiation, toxins and infectious agents should be examined in the future with the growing of more compatible data. Fifth, while XPC rs2228001 polymorphism may contribute to carcinoma, the combined effects of multiple genes or environmental factors might predominate in the development or metastasis of carcinoma [34]. Sixth, the meta-analysis was mainly based on unadjusted estimates. A more precise analysis could be conducted if the individual data were available [35]. Despite these limitations, the present pooled analysis has some key advantages compared with the individual studies. First, a substantial number of cases and controls were extracted from the various studies, which can significantly increase statistical power. Second, the quality of case-control studies included in this analysis was satisfactory based on our selection criteria. Third, no obvious publication bias was detected, suggesting that the results are relatively stable and did not influence the results of our meta-analysis.

In conclusion, this meta-analysis demonstrated that XPC rs2228001 A/C polymorphism may contribute to the risk of developing bladder cancer in the Asian population, but not with other descendants. Furthermore, an elevated risk of bladder cancer was significantly associated with an interaction between XPC rs2228001 polymorphism and smoking status. Future well-designed large studies, particularly referring to gene-environment interactions, are warranted. These future studies should lead to a more comprehensive understanding of the association between XPC rs2228001 A/C polymorphism and bladder cancer risk.

Disclosure of conflict of interest

None.
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