DNA repair gene XRCC1 and XPD polymorphisms and risk of lung cancer in a Chinese population

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X-ray repair cross-complementing group 1 (XRCC1) and xeroderma pigmentosum group D (XPD) are mainly involved in base excision repair (BER) and nucleotide excision repair (NER) of DNA repair pathways, respectively. Polymorphisms of DNA repair gene XRCC1 and XPD has recently been identified, and there is a growing body of evidence that these polymorphisms may have some phenotypic significance. To investigate the role of XRCC1 polymorphisms (codon 194 and codon 399) and XPD polymorphism (codon 751) in lung cancer, a population-based case-control study of 109 lung cancer patients and 109 healthy control subjects (individually matched on age and gender) in a Chinese population was conducted. XRCC1 and XPD genotypes were identified using PCR–restriction fragments length polymorphism technique. Conditional logistic regression analysis revealed that XRCC1 codon 194 Trp/Trp genotype was associated with a borderline increased risk of lung cancer [adjusted odd ratio (OR) = 3.06; confidence interval (CI) 0.94–9.92]. The XPD 751 Lys allele (combined Lys/Lys and Lys/Gln genotypes) was associated with a significantly increased risk of lung cancer (OR = 3.19; CI 1.01–10.07). The risk of lung cancer increased more than additive interaction (adjusted OR = 8.77; CI 1.47–52.31) for the individuals with both putative high-risk genotypes of XRCC1 194 Trp/Trp and XPD 751 Lys allele. Our results suggested that the genotypes of XRCC1 194 Trp/Trp and XPD 751 Lys allele might be the risk genotypes for lung cancer in Chinese population.

Introduction

In recent years, lung cancer has become the leading cause of cancer deaths for both men and women in China. The incidence and death rate of lung cancer in urban populations have reached the number one among malignant tumors (1). It would worsen if smoking rates continue their upward trend (2). Cigarette smoking accounts for an estimated 30% of all cancer deaths and >80% of deaths from lung cancer (3,4). Cigarette smoke contains a myriad of genotoxicants and carcinogens, including oxidants which inflict oxidative DNA damages, and the polycyclic aromatic hydrocarbons (PAH), such as benzo[a]pyrene (B[a]P) which could be bioactivated in vivo into benzo[a]pyrene-diol-epoxide (BPDE) and then irreversibly damage DNA by covalent binding or oxidation (5). While the removal or repair of DNA damage plays a key role in protecting the integrity of the genome from the insults of cancer-causing agents. Individuals with DNA repair defects might be at a higher risk of cancer (6–8). Some studies have reported significantly reduced DNA repair capacity (DRC) in the patients of lung cancer and squamous cell carcinoma of the head and neck compared with the control population (5,9–11).

A complex system of DNA repair enzymes has a vital role in protecting the genome of the cell from carcinogenic exposure. The DNA repair enzyme X-ray repair cross-complementing group 1 (XRCC1) is thought to be involved in the base excision repair (BER) of oxidative DNA and single strand breaks repair (12). XRCC1 protein is proposed to interact with poly(ADP-ribose) polymerase and DNA ligase III in recognition and re-joining of DNA strand breaks, and with DNA polymerase β in BER (12–14). Phenotype studies demonstrated that early embryonic lethality was associated with knockout of mouse XRCC1 (12,15). XRCC1 mutants display sensitivity to alkylating agents and ionizing radiation and exhibit elevated levels of sister chromatid exchange in the Chinese hamster ovary cell lines (16,17). Such alterations could be associated with increased cancer risk.

The xeroderma pigmentosum group D (XPD) protein is an evolutionarily conserved helicase, a subunit of transcription factor II H (TFIIH) that is essential for transcription and nucleotide excision repair (NER) (18). Mutations in XPD prevent its protein product from interacting with p44, another subunit of TFIIH and reduce its heliase activity, resulting in a defect in NER (19). Studies have shown that XPD is involved in repairing genetic damage induced by carcinogens from cigarette smoke (9).

Shen et al. (20) have identified three polymorphisms of the XRCC1 gene and three polymorphisms of the XPD gene, which resulted in amino acid changes at evolutionarily conserved regions. The functional effects of the polymorphisms in XRCC1 and XPD are not well known. It will be interesting to determine if the function of XRCC1 and XPD is significantly modified by these amino acid changes, resulting in deficient DNA repair that may be responsible for increased cancer susceptibility.

The earlier studies have reported the relationship of XRCC1 polymorphisms at codon 194 and codon 399 and cancer risk or carcinogen–DNA adducts (21,22). Recently, XRCC1 polymorphisms in relation to lung cancer risk in Korean, African-Americans and Caucasians were observed (23,24). Lunn et al. (25) have shown the frequency distributions of these two polymorphisms of XRCC1 varied remarkably in Caucasians and in Taiwanese. Moreover, several studies showed that XPD codon 751 polymorphisms were associated with lung cancer, basal cell carcinoma and melanoma susceptibility in western population (11,26–29). As about 700 million Chinese people are subject to smoking directly or indirectly

Abbreviations: BER, base excision repair; DRC, DNA repair capacity; NER, nucleotide excision repair; XPD, xeroderma pigmentosum group D; XRCC1, X-ray repair cross-complementing group 1.
(30), which contributes to lung cancer risk, it is indispensable to investigate the effects of the polymorphisms of XRCC1 and XPD on lung cancer susceptibility in a Chinese population, which has not been reported so far. In this report, we describe a case-control study of lung cancer in a Chinese population to explore the contribution of two polymorphic sites of the XRCC1 gene and one of XPD gene to test the hypothesis that genetic polymorphisms of the two genes contribute to host susceptibility to lung cancer.

Materials and methods

Study subjects

In this case-control study, the case group consisted of 109 diagnosed patients (between March 1994 and September 1997) with histologically confirmed lung carcinoma in the Department of Thoracic Surgery at Jiangsu Cancer Hospital, China. Patients with secondary and recurrent tumors were excluded. The control group comprised 109 healthy volunteers. They were obtained from community centers, cancer-screening programs, and were individually matched to the cases by age, gender and smoking status. Data on age, gender, smoking status and amount were derived from questionnaires (Table I). To be matched to the cases by age, gender and smoking status. The distribution of genotypes of GSTM1 independently affect the genetic polymorphisms of the two genes contribute to host susceptibility to lung cancer.

Polymorphisms of XRCC1 codon 194, codon 399 and XPD codon 751

The putative risk genotypes were further examined by using different reference groups and combinations (Table II). In our study subjects, the distributions of smoking amount (pack-years) were significantly different in cases and controls. Furthermore, our previous study (33) has shown that the polymorphisms of GSTM1 independently affect the genetic susceptibility to lung cancer in the same population. Therefore, we included GSTM1 genotype and pack-years in our final conditional logistic regression model. As shown in Table II, when the XRCC1 194 Arg/Trp and XPD codon 751 Gln/Gln were similar in cases and in controls (42.7 versus 39.2% and 43.1 versus 44.0%, respectively), the frequencies of the XRCC1 codon 194 Arg/Arg (wild-type), Arg/Gln (heterozygotes) and Gln/Gln (mutant) were similar between cases and controls. Given the putative risk genotypes (194 Trp/Trp and 751 Lys allele), there were three possible combined genotypes: high-risk genotypes (XRCC1 194 Trp/Trp and XPD 751 Lys allele), median-risk genotypes (Arg allele and Lys allele, or Trp/Trp and Gln/Gln), and protective genotypes (194 Arg allele and 751 Gln/Gln) that could be considered for further risk assessment in conditional logistic regression.

Results

As shown in Table I, the study included 109 cases and 109 controls. Effort was made to match cases and controls by age, gender and smoking status. The distribution of genotypes of XRCC1 and XPD, and the association between the two XRCC1 and one XPD variants and risk of lung cancer were summarized in Table II. The frequency of XRCC1 codon 194Trp/Trp (mutant genotype) was 10.7% in cases and 4.9% in controls, suggesting that this genotype may be a risk genotype for lung cancer. The frequency of the XPD 751 Gln/Gln (mutant genotype) was 31.7% in cases and 40.3% in controls, suggesting that this genotype may be a protective genotype for lung cancer. The frequencies of the heterozygotes of XRCC1 codon 194 Arg/Trp and XPD codon 751 Lys/Gln were similar in cases and in controls (42.7 versus 39.2% and 43.1 versus 44.0%, respectively). The frequencies of the XRCC1 codon 399 Arg/Arg (wild-type), Arg/Gln (heterozygotes) and Gln/Gln (mutant) were similar between cases and controls. Given the putative risk genotypes (194 Trp/Trp and 751 Lys allele), there were three possible combined genotypes: high-risk genotypes (XRCC1 194 Trp/Trp and XPD 751 Lys allele), median-risk genotypes (Arg allele and Lys allele, or Trp/Trp and Gln/Gln), and protective genotypes (194 Arg allele and 751 Gln/Gln) that could be considered for further risk assessment in conditional logistic regression.

Combination of XRCC1 codon 194 and XPD codon 751 polymorphisms

The combined effects of the polymorphisms in XRCC1 codon 194 and XPD codon 751 on lung cancer risk were further
DNA repair gene *XRCC1* and XPD polymorphisms

### Table II. DNA repair gene XRCC1 and XPD polymorphisms and relative risk of lung cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n = 109) (%)</th>
<th>Controls (n = 109) (%)</th>
<th>P valuea</th>
<th>Adjusted OR (95% CI)b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>XRCC1 codon 194</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg (reference)</td>
<td>48 (46.6)</td>
<td>57 (55.9)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Arg/Trp</td>
<td>44 (42.7)</td>
<td>40 (39.2)</td>
<td>1.55 (0.80–3.02)</td>
<td></td>
</tr>
<tr>
<td>Trp/Trp</td>
<td>11 (10.7)</td>
<td>5 (4.9)</td>
<td>1.65 (0.48–5.64)</td>
<td></td>
</tr>
<tr>
<td>Arg/Arg + Arg/Trp (reference)</td>
<td>92 (89.3)</td>
<td>97 (95.1)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Trp/Trp</td>
<td>11 (10.7)</td>
<td>5 (4.9)</td>
<td>3.06 (0.94–9.92)</td>
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</tr>
<tr>
<td>Missing</td>
<td>6</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele frequency (Trp)</td>
<td></td>
<td></td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td><strong>XRCC1 codon 399</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg (reference)</td>
<td>55 (53.4)</td>
<td>52 (52.5)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>43 (41.7)</td>
<td>40 (40.4)</td>
<td>0.97 (0.51–1.84)</td>
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<tr>
<td>Gln/Gln</td>
<td>5 (4.9)</td>
<td>7 (7.1)</td>
<td>0.32 (0.03–3.16)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>6</td>
<td>10</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Allele frequency (Gln)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>XPD codon 751</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln/Gln (reference)</td>
<td>11 (10.1)</td>
<td>20 (18.3)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Lys/Gln</td>
<td>47 (43.1)</td>
<td>48 (44.1)</td>
<td>2.24 (0.37–13.65)</td>
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<tr>
<td>Lys/Lys</td>
<td>51 (46.8)</td>
<td>41 (37.6)</td>
<td>13.58 (1.25–147.65)</td>
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<tr>
<td>Lys/Gln + Lys/Lys</td>
<td>98 (89.9)</td>
<td>89 (81.7)</td>
<td>3.19 (1.01–10.07)</td>
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</tr>
<tr>
<td>Allele frequency (Gln)</td>
<td>31.7%</td>
<td>40.3%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aTwo-side χ² test.  
bConditional logistic regression analysis. Adjusted for pack-years and GSTM1 genotype.

### Table III. Combined analysis of XRCC1 codon 194 and XPD codon 751 polymorphisms in relation to lung cancer

<table>
<thead>
<tr>
<th>XRCC1-194</th>
<th>XPD-751</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Adjusted OR (95% CI)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg alleleb + Gln/Gln</td>
<td>8</td>
<td>18</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>or Trp/Trp + Gln/Gln</td>
<td>86</td>
<td>79</td>
<td>3.71 (0.97–14.16)</td>
<td></td>
</tr>
<tr>
<td>Arg alleleb + Lys allelec + Lys/Gln</td>
<td>9</td>
<td>5</td>
<td>8.77 (1.47–52.31)</td>
<td></td>
</tr>
</tbody>
</table>

aConditional logistic regression analysis. Adjusted for GSTM1 genotype and pack-years.  
bCombination of XRCC1 codon 194 Arg/Arg and Arg/Trp.  
cCombination of XRCC1 codon 751 Gln/Lys and Lys/Lys.

explored. The additive interaction model revealed that carrying either risk genotype, XRCC1 194 Trp/Trp or XPD 751 Lys allele, contributed to a borderline increased risk of lung cancer (OR = 3.71; CI 0.97–14.19). Individuals with both risk genotypes have a higher elevated risk of lung cancer (OR = 11.04; CI 1.20–101.59). No statistically significant association of polymorphisms of XRCC1 codon 194 and XPD codon 751 and lung cancer risk was found when stratifying the smoking amount and histological type of cases (Table IV).

### Stratification of XRCC1 codon 194 and XPD codon 751 polymorphisms to lung cancer risk

After stratifying for age, gender, smoking amount and histological types of cases, we found that the 751 Lys allele genotypes were associated with a significantly increased risk in younger group (less than median of age 58) (OR = 3.06; CI 0.94–9.92). We suggested that it might be due to the genetic trait differences. We found the frequencies of XRCC1 codon 194 Trp allele were significantly higher (24.5%) and XPD1 codon 399 Gln was much less (27.3%) in our study than those in Caucasians (~5 and 35%, respectively) (21,25,36). These different frequencies of codon 194 and codon 399 may account for their different contributions to lung cancer risk.

### Discussion

There has been ample epidemiological evidence to indicate that not all individuals similarly exposed to cigarette smoke eventually develop lung cancer. The prevailing concept is that defects in one or more steps of DNA repair may be an important determinant in carcinogenesis.

DNA sequencing has identified three non-conservative changes in human alleles of XRCC1. Most studies have found that XRCC1 codon 399 polymorphism, instead of codon 194 polymorphism, was related to the risk to cancer in western population studies (20,25,34,35). However, there was no evidence observed in our study that XRCC1 codon 399 Gln allele was associated with lung cancer risk in a Chinese population. In contrast, we found that the 194 Trp/Trp genotype was associated with a borderline increased risk of lung cancer (OR = 3.06; CI 0.94–9.92). There was no statistically significant association of polymorphisms of XRCC1 codon 194 and XPD codon 751 and lung cancer risk when stratifying the smoking amount and histological type of cases (Table IV).
(29) reported no association of the XPD codon 751 polymorphism to lung cancer risk need to be further explored in a larger Chinese population. The results suggested that the individuals with both XRCC1 194 Trp/Trp and XPD 751 Lys allele genotypes seemed to synergistically increase the risk of lung cancer, compared with those with either of them. Because most environmental exposures including cigarette smoke are complex mixed substances, it is plausible that the repair of DNA damage intrigued by these mixed substances need either BER pathway or NER pathway. The failure or diminished DRC on either side may cause the cancer risk. If the failure or diminished DRC happen on both sides, they may both contribute to the elevated risk of cancer.

After stratification for variables, i.e. age, gender, smoking amount and histological type of cases (Table IV), the XRCC1 194 Trp/Trp genotype was found associated with a significantly increased risk of lung cancer in male group rather than in female group. We noted that all female subjects (total 38) are non-smokers, and the lack of smoking exposure may give little chance to reveal the effect of XRCC1 codon 194 polymorphism. David-Beabes and London (24) observed somewhat decreased risk of lung cancer for the African-American and Caucasian subjects in Los Angeles County, smoking 20+ cigarettes/day and carrying codon 194 Trp alleles. But we could not find whether cigarette consumption may modify the risk of lung cancer, due to insufficient subjects in the smoking amount subgroups. The XPD 751 Lys allele was associated with elevated risk of lung cancer in younger group, who tended to be more liable to the subtle XPD codon 751 genotypic differences after relatively minor environmental exposures to carcinogens. But again, our results may be biased by the relatively small number of subjects in the various subgroups and, therefore, limited the interpretation of this founding. Further studies with larger samples and more complete measures of tobacco exposure (PAH-adducts) would be needed to confirm these preliminary findings.

In brief, to our knowledge, this is the first report of XRCC1 and XPD polymorphisms in relation to lung cancer risk in a case-control study in Chinese population. The results suggested that XRCC1 194 Trp/Trp and XPD 751 Lys allele might be the risk genotypes for lung cancer in Chinese population. Further mechanistic studies of these proteins are needed to enhance our ability to identify those individuals most susceptible to lung carcinogenesis.

Acknowledgement
We thank Dr Andrew Rundle for his helpful discussion.

References

### Table IV. Stratification analysis of XRCC1 codon 194 and XPD codon 751 polymorphisms in relation to lung cancer by age, gender and smoking (pack-years) and histological type of cases

| Variable | No. of cases | No. of controls | Adjusted OR (95% CI)*
|----------|--------------|----------------|----------------------
| Histological type | | | |
| Adenocarcinoma | XRCC1 194 Trp/Trp | 8 | 2 | 2.90 (0.74–11.31)
| XPD 751 Lys allele | 46 | 42 | 3.51 (0.62–19.86)
| Squamous cell carcinoma | XRCC1 194 Trp/Trp | 3 | 3 | 3.10 (0.32–30.48)
| XPD 751 Lys allele | 42 | 36 | 6.83 (0.80–58.45)
| Age (in years) | | | |
| <58 | XRCC1 194 Trp/Trp | 5 | 1 | 8.00 (0.88–88.52)
| XPD 751 Lys allele | 53 | 45 | 11.04 (1.20–101.59)
| XRCC1 194 Trp/Trp | 6 | 4 | 1.95 (0.47–8.14)
| XPD 751 Lys allele | 45 | 44 | 1.47 (0.29–7.35)
| Gender | | | |
| Female | XRCC1 194 Trp/Trp | 2 | 2 | 0.85 (0.13–5.83)
| XPD 751 Lys allele | 18 | 16 | 2.73 (0.26–29.09)
| Male | XRCC1 194 Trp/Trp | 9 | 3 | 8.19 (1.30–51.74)
| XPD 751 Lys allele | 80 | 73 | 3.36 (0.89–12.65)
| Smoking amount | | | |
| Pack-years = 0 | XRCC1 194 Trp/Trp | 3 | 2 | 0.81 (0.12–5.34)
| XPD 751 Lys allele | 28 | 25 | 4.66 (0.46–47.73)
| Pack-years >, <30 | XRCC1 194 Trp/Trp | 3 | 2 | N/A
| XPD 751 Lys allele | 29 | 29 | N/A
| Pack-years ≥30 | XRCC1 194 Trp/Trp | 5 | 1 | 3.32 (0.30–36.71)
| XPD 751 Lys allele | 41 | 35 | 1.03 (0.06–16.76)

*Conditional logistic regression analysis. Adjusted for GSTM1 genotype and pack-years.

size of our study was marginally adequate, and the mechanisms of XRCC1 codon 194 polymorphism to lung cancer risk need to be further explored in a larger Chinese population.

XPD codes for a DNA helicase involved in transcription and NER. Rare XPD mutations result in genetic diseases, such as xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy (37). XPD polymorphism may also act as a genetic susceptibility factor. Our results suggested that the XPD codon 751 Lys allele contributed to the increased risk of lung cancer (OR = 3.19; CI 1.01–10.07). The amino acid substitution Lys → Gln, which is a change from a basic to a polar amino acid, could possibly improve the function of the XPD protein (20,27), and thus acting as protective. Recently Lunn et al. (32) reported that possessing the XPD codon 751 Lys/Lys (wild-type genotype) was associated with an increased risk of exhibiting chromatid aberrations in human lymphocytes. Dybdahl et al. (27) and Tomescu et al. (26) also reported that individuals with the XPD codon 751 Lys/Lys genotype were at higher risk of basal cell carcinoma and melanoma, respectively. On the other hand, Sturgis et al. (28) reported that the XPD 751 Gln/Gln (mutant genotype) was associated with a borderline increased risk for upper aerodigestive tract cancer. Spitz et al. (11) reported that the XPD 751 Gln/Gln genotype was associated with less optimal DRC, while Moller et al. (29) reported no association of the XPD codon 751 polymorphism with DRC. Clearly, it is difficult to detect subtle differences in DRC due to a single polymorphism of a single gene in a very complex pathway. The inconsistency in the effect of XPD polymorphism at codon 751 in these studies may be also ascribed to the exposure and interaction with other genes participating in DNA damage recognition, repair and cell cycle regulation (18).

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References
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