·Basic Research·

# Correlation of XPD gene with susceptibility to gastric cancer

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**[Abstract] Background and Objective:** Mutations in DNA repair system are related to carcinogenesis. This study was to evaluate the correlations of polymorphisms and haplotypes of XPD gene with individual susceptibility to gastric cancer. **Methods:** Genomic DNA were extracted from peripheral blood leukocytes of 207 gastric cancer patients and 212 healthy controls. Genotypes at codon 312 and codon 751 polymorphic sites were identified by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) or polymerase chain reaction-restriction fragment length polymorphism (PCR-PFLP), respectively. **Results:** At codon 312, the frequency of GA or AA genotype was higher in the gastric cancer patients than in the healthy controls ($P$<0.01, OR=3.41, 95% CI: 2.06–4.79; $P$<0.01, $OR$=3.47, 95% CI: 1.39–8.68). No significant difference was found in the distribution of the polymorphism at codon 751 between the two groups ($P$>0.05). By the haplotype AA (codon 312A-codon 751A) analysis, the frequency of heterozygote (–/AA) or homozygote (AA/AA) was higher in the patients than in the controls ($P$<0.01, $OR$=2.81, 95% CI: 1.82–4.34; $P$=0.02, $OR$=3.92, 95% CI: 1.31–11.70, respectively). Whereas there were no significant differences of the other three haplotypes between the patients and the controls ($P$>0.05). **Conclusions:** The polymorphism of XPD at codon 312 might contribute to the etiology of gastric cancer. The haplotype AA (codon 312A-codon 751A) would be a critical risk factor of the susceptibility to gastric cancer.

**Key words:** gastric neoplasm, XPD gene, polymorphism, haplotype, etiology

Recent studies have identified that mutations in DNA repair system reduce DNA repair capacity, and increase the risk of carcinogenesis.[1] Nucleotide excision repair (NER) is recognized as the most important DNA damage repair pathway.[2] The xeroderma pigmentosum complementary group D (XPD) protein is an ATP-dependent DNA helicase that is associated with transcription factor Ⅱ H (TF Ⅱ H) complex and anchors cdk-activating kinase (CAK) to the core TF Ⅱ F.[3] Therefore, XPD plays a role in NER pathway and RNA polymerase Ⅱ-mediated transcription,[4] and takes parts in cell apoptosis by interacting with p53.[5] The activity of XPD is essential for carcinogenesis, and loss of XPD function leads to embryonic lethality.[6]

XPD, also called excision repair cross complement gene (ERCC), is located on human chromosome 9 with a length of 5.4 kb. Eight single nucleotide polymorphisms in the human XPD coding region have been described.[7] Codon 312 (exon 10G→A, Asp→Asn) and codon 751 (exon 23A→C, Lys→Gln) are the most common polymorphisms. The correlations of XPD polymorphisms to head and neck squamous cell carcinoma, lung squamous cell carcinoma, basal cell carcinoma, breast cancer and esophageal cancer have been widely explored,[8-12] while their correlations to susceptibility of gastric cancer have seldom been reported.[12-15] Moreover, the results are inconsistent. Thus, we carried out a case-control study in north China to explore the correlations of the polymorphisms of codon 312G/A and codon 751A/C to the susceptibility to gastric cancer.

## Materials and Methods

### Study subjects

A total of 207 out-patients or in-patients with gastric cancer treated in Peking University First Hospital (105 cases) and Qianfoshan Hospital in Shandong (102 cases) from 2004 to 2007 were confirmed by gastroendoscopy or surgical excision biopsy. These patients, including 94 men and 113 women, ranged in age of 39-69, with a median of 50. The control group included 212 healthy blood donors from the same area (112 in Beijing, 100 in Shandong), without kinship, past history of tumor and autoimmune diseases. These controls, including 102 men and 110 women, ranged in age of 36–67, with a median of 49. Informed consent forms were signed by all subjects. The demographic information, such as age, sex, history of smoking

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and drinking, are listed in Table 1.

**Table 1 General characteristics of two groups**

| Variables | cases [number(%)] | controls [number(%)] | *P* |
|---|---|---|---|
| Median age (range,years) | 50(39–69) | 49(36–67) | 0.29[a] |
| Gender | | | |
| Male | 94(45.4) | 102(48.1) | 0.58[b] |
| Female | 113(54.6) | 110(51.9) | |
| Smoking | | | |
| Yes | 66(31.9) | 55(25.9) | 0.18[b] |
| No | 141(68.1) | 157(74.1) | |
| Drinking | | | |
| Yes | 58(28.0) | 63(29.7) | 0.70[b] |
| No | 149(72.0) | 149(70.3) | |

[a]Mann-Whitney *U* test; [b]chi-square test.

### DNA extraction

Genomic DNA was extracted from peripheral blood by phenol/chloroform and precipitated by absolute ethanol, and stored at -20℃.

### XPD polymorphisms identification

Genotype at codon 312 was identified by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).[16] The forward primer for wild type G was 5'-GTCGGGGCTCACCCTGCAGCACTTCGGC-3'; the forward primer for mutant type A was 5'-GTCGGGGCTCACCCTGCAG-CACTTCGAT- 3', and the reverse primer was 5'-CAGGATCAAA-GAGACAGACGAGCAGCGC-3'. All primers were synthesized by Beijing AuGCT Biotechnology Co., Ltd. The product size was 150 bp. Each sample was amplified twice by different pairs of primers. A standard 25 μL PCR reaction system contained about 100–150 ng of template DNA, 0.5 μL of forward primer (10 μmol/L), 0.5 μL of reverse primer (10 μmol/L), 0.5 μL of dNTP (10 mmol/L), 1.5 U of *Taq* DNA polymerase, 2.5 μL of 10× buffer and 1.5 μL of $MgCl_2$ (25 mmol/L). The cycling conditions for amplification were as follows: pre-denaturation at 94℃ for 2 min; then 38 cycles of denaturation at 94℃ for 30 s, annealing at 66.5℃ (wild type) or 62.5℃ (mutant type) for 50 s, and extension at 72℃ for 1 min; followed by a final extension at 72℃ for 5 min. For internal controls, the forward primer was 5'-CCC-ACCTTCCCCTCTCTCCAGGCAAATGGG-3' and the reverse primer was 5'-GGGCCTCAGTCCCAACATGGCTAAGAGGTG-3'. The product size was 360 bp. All PCR products were run on 2% agarose gels.

Genotype at codon 751 was identified by using polymerase chain reaction-restriction fragment length polymorphism (PCR-PFLP).[17] The primers were 5'-TCAAACATCCTGTCCCTACT-3' and 5'-CTGCGATTAAAGGCTGTGGA-3', the annealing temperature was 62℃ and the product size was 344 bp. The product was digested with *Pst* Ⅰ (NEB Company) in a 20 μL reaction system containing 10 μL of PCR product, 6 U of *Pst* Ⅰ, 2.0 μL of 10× buffer and 0.2 μL of 100× BSA, and incubated at 37℃ overnight. The digestion products were separated on a 3% agarose gel. 10% of the PCR products with certain genotypes were sequencied by Beijing Genomics Institute.

### Statistical analysis

SPSS13.0 software was used for statistical analysis. Age distribution between the patients and controls was compared by Mann-Whitney test. Distributions of gender, smoking and drinking, and genotype frequency compliance with Hardy-Weinberg equilibrium were analyzed by $\chi^2$-test. Adjusted odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by unconditional logistic regression analyses to evaluate the correlation of genotypes to gastric cancer. PHASE 1.0 software was used to construct haplotypes. The correlation of haplotypes to gastric cancer was notated by the values of OR and 95% CI in unconditional Logistic regression analyses. All statistical tests were bilateral probability tests. A value of $P<0.05$ was considered significant.

## Results

### XPD polymorphisms

The genotypes at codon 312 were identified through specific fragments amplified by different primer pairs (Fig. 1). The genotypes at codon 715 were identified by the location and number of digestion products on agarose gels (Fig. 2). The sequencing results are shown in Figure 3.

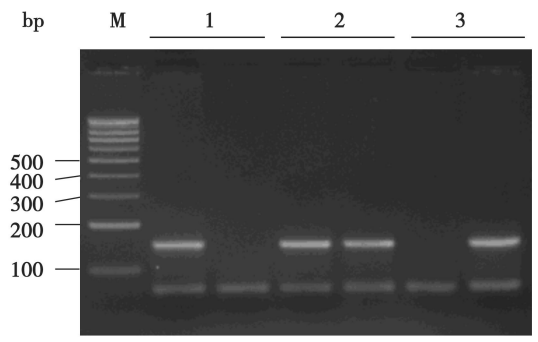


Figure 1 Electrophoregram of three genotypes at codon 312
Lane M, marker; Lane 1, genotype GG; Lane 2, genotype GA; Lane 3, genotype AA.

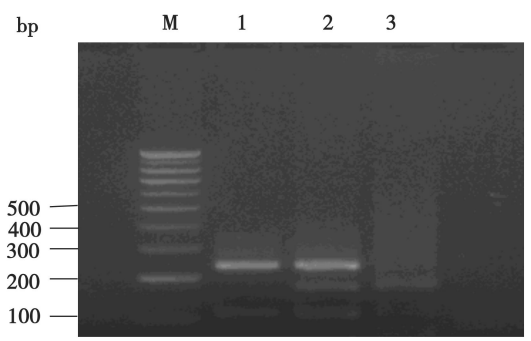


Figure 2 Electrophoregram of three genotypes at codon 751
Lane M, marker; Lane 1, genotype AA, 234 bp+110 bp; Lane 2, genotype AC, 234 bp+171 bp+110 bp+63 bp; Lane 3, genotype CC, 171 bp+110 bp+63 bp.

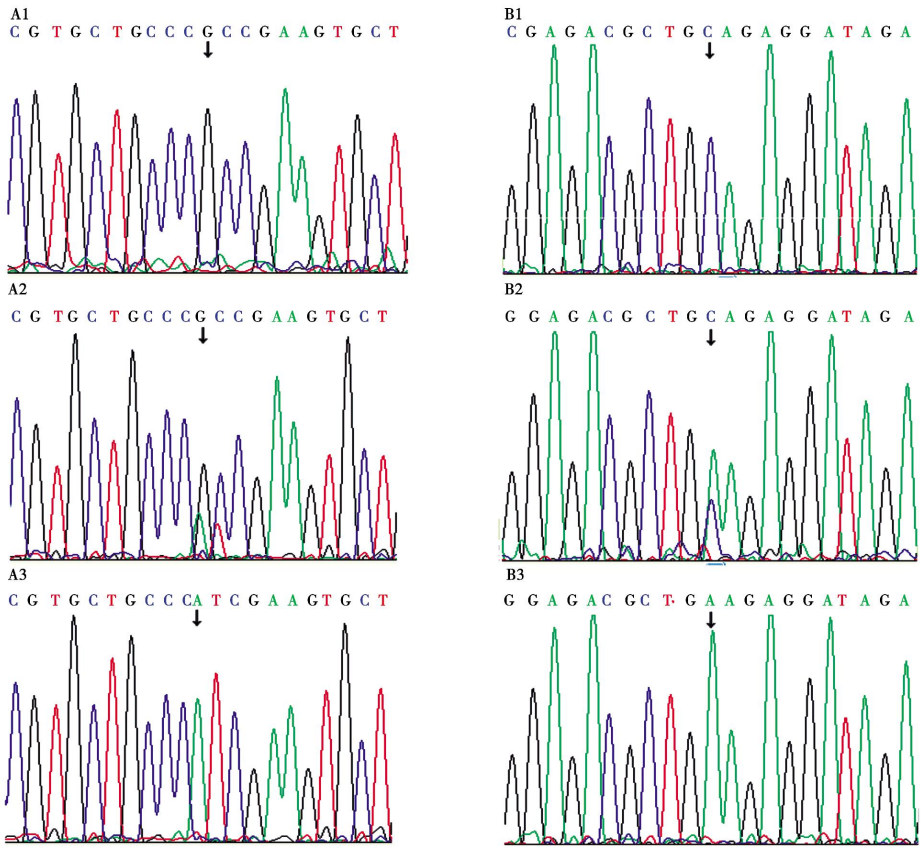


Figure 3 Sequencing of genotypes at codon 312 and codon 751

A1, genotype GG at codon 312; A2, genotype GA at codon 312; A3, genotype AA at codon 312; B1, genotype AA at codon 751; B2, genotype AC at codon 751; B3, genotype CC at codon 751.

## Characteristics of the study populations

The differences in distribution of age, gender, smoking and drinking between the patients and controls were not significant ($P$>0.05).

## Correlation of XPD polymorphisms to the risk of gastric cancer

As shown in Table 2, the distribution of genotype frequencies was compliable with Hardy-Weinberg equilibrium ($P$>0.05). The proportions of genotypes GA and AA at codon 312 were significantly higher in gastric cancer patients than in controls ($P$<0.01). The risk of gastric cancer in the individuals with genotype GA or AA was 3.41 or 3.47 folds of that in the individuals with genotype GG. The distribution of three genotypes at codon 751 between the two groups was not significant ($P$> 0.05).

**Table 2 Correlation of XPD gene polymorphisms with the susceptibility gastric cancer**

| Variables | Cases [number(%)] | Controls[number(%)] | $P$[a] | OR(95% CI)[a] |
|---|---|---|---|---|
| codon 312G/A | | | | |
| GG | 75(36.2) | 132(62.3) | - | 1.00 |
| GA | 117(56.5) | 72(34.0) | <0.01 | 3.41(2.06-4.79) |
| AA | 15(7.2) | 8(3.8) | <0.01 | 3.47(1.39-8.68) |
| codon 751A/C | | | | |
| AA | 166(80.2) | 172(81.1) | - | 1.00 |
| AC | 39(18.8) | 39(18.4) | 0.96 | 1.01(0.61-1.67) |
| CC | 2(1.0) | 1(0.5) | 0.66 | 1.72(0.15-19.50) |

[a]Logistic regression model, adjusted by age, gender, alcohol consumption, and smoking.

## Construction of haplotypes and their correlations to the risk of gastric cancer

We constructed four haplotypes (GA, AA, AC, and GC) according to the polymorphisms of codon 312 and codon 751. The proportion of haplotype AA (codon 312A-codon 751A) was higher in gastric cancer patients than in controls (28.0% vs. 15.1%). The proportions of heterozygote (-/AA) and homozygote (AA/AA) were significantly higher in gastric cancer patients than in controls ($P$<0.05). Heterozygote (-/AA) and homozygote (AA/AA) significantly increased the risk of gastric cancer compared to the others. By similar analyses, the differences in the distribution of the other three haplotypes between two groups were not significant ($P$>0.05) as shown in Table 3.

# Discussion

The correlation of the polymorphisms of candidate tumor susceptibility gene XPD to carcinogenesis has become a hot spot, but their correlation to the risk of gastric cancer has seldom been explored, and the results are controversial. In the present

**Table 3 Correlation of four haplotypes with the susceptibility to gastric cancer**

| Haplotypes | Cases[number(%)] | Controls[number(%)] | P[a] | OR(95% CI)[a] |
|---|---|---|---|---|
| GA | | | | |
| -/-[b] | 16(7.7) | 8(3.8) | - | 1.00 |
| -/GA | 127(61.4) | 89(42.0) | 0.54 | 0.75(0.31-1.87) |
| GA/GA | 64(30.9) | 115(54.2) | <0.01 | 0.27(0.11-0.68) |
| AA | | | | |
| -/- | 103(49.8) | 153(72.2) | - | 1.00 |
| -/AA | 92(44.4) | 54(25.5) | <0.01 | 2.81(1.82-4.34) |
| AA/AA | 12(5.8) | 5(2.4) | 0.02 | 3.92(1.31-11.70) |
| AC | | | | |
| -/- | 177(85.5) | 189(89.2) | - | 1.00 |
| -/ AC | 29(14.0) | 22(10.4) | 0.26 | 1.41(0.77-2.56) |
| AC/AC | 1(0.5) | 1(0.5) | 0.99 | 0.98(0.06-15.96) |
| GC | | | | |
| -/- | 195(94.2) | 195(92.0) | - | 1.00 |
| -/GC | 12(5.8) | 17(8.0) | 0.28 | 0.65(0.30-1.42) |
| GC/GC | 0(0.0) | 0(0.0) | - | - |

[a]Logistic regression model, adjusted by age, gender, alcohol consumption, and smoking; [b]-, denotes any other haplotype, for example, -/GA indicates the GA haplotype in combination with any other haplotype.

study, we found that the polymorphism of XPD codon 312 was associated with the initiation of gastric cancer. The frequencies of genotypes GA and AA were significantly higher in gastric cancer patients than in controls ($P$<0.01). The individuals with genotype GA or AA increased risk of gastric cancer as 3.41 or 3.47 folds of those with genotype GG, indicating that genotype GA or AA at codon 312 was related to the susceptibility to gastric cancer. Tang et al.[11] and Hemminki et al.[18] found that DNA repair capacity with allele A at codon 312G/A was very low. Since codon 312 of XPD is highly conserved through evolution, suggesting codon 312 may play a role in maintaining the function of XPD protein, its mutation would affect DNA repair capacity, transcriptional activity and cell apoptosis.

Our results showed that the polymorphism at codon 751 was not associated with the susceptibility to gastric cancer ($P$>0.05). Codon 751 is located in the carboxy-terminal of XPD gene, which is the site for interaction with p44 to enhance its helicase activity, but it is not evolutionarily conserved. It is speculated that the polymorphism of codon 751 may affect the efficiency of DNA repair.[19] This is inconsistent with other reports.[8,10,18] Different tumor locations may mainly contribute to the inconsistence.

Huang et al.,[13] Ye et al.,[12] and Ruzzo et al.[14] found that the polymorphisms of codon 312 and codon 751 did not had clear association with gastric cancer in Polish, Swedish and Italian. Lou et al.[15] got different results in the northeast Chinese population, suggesting that the difference in the correlation of XPD to the susceptibility to gastric cancer might be due to ethnic differences.

For tumor susceptibility genes, the polymorphisms may not only play isolated roles but also have interactions. They can be combined to construct haplotypes. Therefore, analyzing haplotypes can reveal more credible associations between the genetic polymorphisms and diseases. We constructed four haploypes at codon 312 and codon 751, and found that the correlations of heterozygote (-/AA) and homozygote (AA/AA) to the risk of gastric cancer were 2.81 and 3.92 folds higher than those of the others, suggesting that haplotype AA could increase the risk of gastric cancer.

Taken together, the polymorphism of XPD codon 312 is associated with the susceptibility to gastric cancer, and haplotype AA (codon 312A-codon 751A) is a risk factor of gastric cancer. Therefore, detecting the polymorphism of XPD codon 312 will be helpful for prevention and early diagnosis of gastric cancer. Furthermore, it is necessary to explore the functions of XPD gene to reveal the mechanisms of its polymorphisms affecting carcinogenesis.

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