Relationship between ERCC2 Polymorphism and Risk of Lung Cancer in Chinese Nonsmoker

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ABSTRACT

Objective: Excision repair cross-complimentary group 2 (ERCC2) is one of the important DNA repair genes. ERCC2 codon 751 polymorphism has been shown to modulate cancer risk. We therefore assessed the relationship between the ERCC2 polymorphism and the susceptibility to lung cancer in nonsmoking females via a hospital-based case-control study. **Methods**: There were 105 lung cancer cases and matched healthy controls in this study. Information concerning demographic and risk factors was obtained, each person donated 2 ml blood for biomarker testing. ERCC2 genotypes were determined by PCR-RFLP method. All of the statistical analyses were performed with SPSS (v 12.0). **Results**: All of the subjects in this study were nonsmoking females in Shenyang. There was significant difference between the frequencies of ERCC2 polymorphism in cancer cases and controls (P<0.05). The frequencies of ERCC2 751 Gln allele were 6.2% in controls and 13.8% in cancer cases. The individuals with Lys/Gln+Gln/Gln combined genotype were at an increased risk for lung cancer as compared with those carrying the Lys/Lys genotype (adjusted OR=2.80, 95%=CI 1.21–6.48). We analyzed the environmental risk factors for lung cancer in nonsmoking females. The cancer patients showed a higher prevalence of exposure to cooking fumes compared with controls (OR=2.44, P<0.05). Furthermore, an interaction between exposure to cooking fumes and the variant ERCC2 751 Gln allele on the risk of lung cancer was observed. Individuals with both risk genotype and exposure to cooking fumes had a higher risk of cancer than those with only one of them. **Conclusion**: The above findings indicate that the genetic polymorphism in the ERCC2 codon 751 is associated with the risk of lung cancer in nonsmoking females.

Key words: **ERCC2 gene; Lung cancer; Nonsmoker**

In recent years, lung cancer has become the leading cause of cancer-related deaths for both men and women in China. The incidence and mortality rate of lung cancer in China urban populations have reached the number one among malignant tumors. Both environmental risk factors and genetic susceptibility play important roles in the development of lung cancer[1]. About the genetic susceptibility to lung cancer, studies focus on polymorphisms in genes involved in carcinogen metabolism and DNA repair[2]. Excision repair cross-complimentary group 2 (ERCC2) is one of the important DNA repair genes. ERCC2 is located in chromosome 19q13.2-13.3 and codes for an evolutionarily conserved helicase, a subunit of TF-II H complex which is essential for transcription and nucleotide excision repair (NER).

One of the common polymorphisms of ERCC2 gene is the A->C polymorphism in exon 23 at nucleotide position 35931 [Lys751Gln]. Although the exact mechanisms how ERCC2 polymorphisms affect cancer risk at the molecular level remain to be unraveled, the published studies imply that these polymorphisms may prevent the protein from interacting with p44 (another subunit of TF II H complex) and decrease helicase activity, resulting in a defect in NER and deficient DNA repair capacity (DRC) that may be responsible for

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increased susceptibility of cancer.

The polymorphisms of DNA repair gene have been proposed as candidate cancer-susceptibility factors. The effect of the ERCC2 polymorphism on lung cancer susceptibility in non-smoking females has not been reported so far. In this study, we described a case-control study of lung cancer in non-smoking female population in Shenyang to test the hypothesis that genetic polymorphism of ERCC2 gene contributes to host susceptibility to lung cancer and explored the interaction between genetic polymorphism and exposure to environmental risk factors in the development of lung cancer.

MATERIALS AND METHODS

Study Population

In this case-control study, the case group consisted of 105 diagnosed nonsmoking female patients (between January 2004 and November 2005) with histological confirmed lung carcinoma at Liaoning Cancer Hospital or 202 Hospital, China. At the same time controls were selected from cancer-free patients with other lung diseases but free of cancer history and symptoms. Controls were matched to cases on age (±5 years). Information concerning demographic characteristics, passive smoking history, exposure to cooking fumes, coom exposure, family history of cancer, occupational exposure and dietary habit was obtained for each case and control by trained interviews. After informed consent was obtained, each person donated 2 ml blood in Heparinized tubes and stored at -80°C for biomarker testing.

ERCC2 Genotyping

An amount of 2 ml whole blood was employed for DNA extraction. Polymerase chain reaction (PCR) followed by enzymatic digestion was used for the genotyping of the ERCC2 Lys751Gln. PCR primers were 5'-GCCCCGCTCTGGATTATACG-3' and 5'- CTATCATCTCCTGGCCCCC-3', which generate a 436 bp fragment. PCR was performed in a total volume of 25 μl containing 1 μg of genomic DNA, 20 ρmol/L of each primer, 2.5 mmol/L deoxynucleotide triphosphates (dNTP), 2.5 μl of 10×PCR buffer (100 mmol/L Tris-HCL pH 8.3, 500 mmol/L KCl and 15 mmol/L $MgCL_2$, and 5 unit of Taq polymerase (TaKaRa Biotechnology Co., Ltd). The PCR reaction was carried out as follow: initial denaturation step at 94°C for 2 min, 30 cycles of 94°C for 30 s, 59°C for 30 s, 72°C for 1 min, followed by a final elongation step at 72°C for 10 min.

The PCR products were digested at 37°C with Pst I (TaKaRa Biotechnology Co., Ltd) overnight. The digestion product was then resolved on 3% agarose gel. The homozygous Lys allele was determined by the presence of two bands at 290 bp and 146 bp, the homozygous Gln allele was determined by the presence of three bands at 227 bp, 146 bp and 63 bp, and the heterozygous Lys/Gln allele was determined by the presence of four bands at 290 bp, 227 bp, 146 bp and 63 bp.

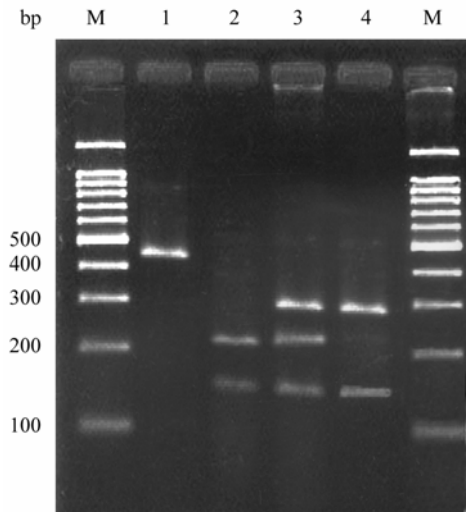

Fig. 1. A representative agarose gel picture of ERCC2 Lys751Gln genotypes
M: markers; Lane 1: the fragment of 436 bp; Lane 2: genotype Gln/Gln; Lane 3: genotype Lys/Gln; Lane 4: genotype Lys/Lys

Statistical Methods

Two-side χ^2 test was used to compare the distribution of the genotypes and risk factors between cancer cases and controls. Unconditional logistic regression analysis was performed to calculate the odds ratios (OR) with 95% confidence intervals (CI) for estimating the association between certain genotypes and lung cancer and exploring the interaction of genetic polymorphism and exposure to environmental risk factors. All of the statistical analyses were performed with SPSS (v 12.0).

RESULTS

The study included 105 cases and 105 matched