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*Original Article*

# A genetic variant in interleukin 8-251A/T is associated with the risk of clear cell renal cell carcinoma in Chinese population

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**Abstract:** Background: Interleukin-8 (IL-8) is an angiogenic chemokine that plays a potent role in both development and progression of many human malignancies. However, there are no data about the role of IL-8 polymorphism in development of RCC. Patients and methods: A hospital-based case-control study was conducted among 520 patients with RCC and 520 healthy controls to investigate the possible association between the IL-8-251A/T and +781C/T polymorphisms respectively, and the risk of RCC. Results: Significant differences of genotype distribution were observed between RCC cases and controls at the IL-8-251T/A genotypes. Compared with the IL-8-251T/A homozygote TT, the heterozygous TA genotype was associated with significantly increased risk for RCC (OR = 1.83, 95% CI = 1.23-3.95, P = 0.019); the AA genotype was associated with increased risk for RCC (OR = 1.88, 95% CI = 1.29-3.68, P = 0.015). TA and AA combined variants were associated with increased risk for RCC compared with the TT genotype (OR = 1.85, 95% CI = 1.24-3.81, P = 0.018). Moreover, the genotype AA of IL-8-251T/A carried a higher risk of RCC metastasis and later stages, compared with the TT genotype. However, the genotype and allele frequencies of IL-8+781C/T polymorphisms in RCC patients were not significantly different from controls. Conclusion: Our results showed that the IL-8-251A/T genotype was associated with increased risk for development and metastasis of RCC in Chinese Han population.

**Keywords:** IL-8, renal cell carcinoma, single-nucleotide polymorphism, risk

## Introduction

Renal cell carcinoma (RCC) represents 2-3% of all cancers and is the third leading cause of death among genitourinary malignancies, with the highest incidence occurring in the developed countries [1]. It is estimated that approximately 37.7 men and 16.6 women per 100000 Chinese individuals are diagnosed with RCC every year [2]. Accumulative epidemiological studies have suggested that cigarette smoking, gender, obesity and a history of hypertension, along with some other less certain factors, such as alcohol consumption, occupational exposures, physical activity and family history of cancer, are associated with RCC [3-5].

Although the exact etiology of RCC remains unclear, studies have shown that it involves environmental and genetic factors. Molecular epidemiology studies suggested that single nucleotide polymorphisms (SNPs) in specific genes and pathways may play an important role in the pathogenesis of RCC. Interleukin-8 (IL8) is a member of the CXC chemokine family [6]. It is a major mediator of inflammation, acting as a chemoattractant for neutrophils, basophils and T-cells, and is a potent angiogenic factor [7]. IL-8 is encoded by the IL-8 gene which was localized to 4q12-q13, consisting of four exons, three introns, and the proximal promoter region [8]. The IL-8 gene polymorphisms at positions -251 (rs4073) and +781 (rs2227306) are known to affect IL-8 expression [9-11].

Previous studies have revealed that SNPs of the IL-8 gene were associated with several diseases, such as respiratory syncytial virus bronchiolitis, acute respiratory distress syndrome and gastric cancer [12, 13]. To the best of our knowl-

**Table 1.** Distribution of selected variables between the renal cell carcinoma cases and control subjects

| Characteristics | Cases (%) N = 520 | Controls (%) N = 520 | *P* value* |
|---|---|---|---|
| Mean Age(years) | 61.5 (± 13.3) | 59.7 (± 12.4) | 0.316 |
| ≤ 60 | 282 (54.2) | 303 (58.3) | |
| > 60 | 238 (45.8) | 217 (41.7) | |
| Gender | | | 0.249 |
| Male | 367 (70.6) | 345 (66.3) | |
| Female | 153 (29.4) | 175 (33.7) | |
| BMI, kg/m$^2$ | 25.5 (± 3.3) | 24.6 (± 3.8) | 0.315 |
| ≤ 25 | 245 (47.1) | 224 (43.1) | |
| > 25 | 275 (52.9) | 296 (56.9) | |
| Smoking status | | | 0.221 |
| Never | 278 (53.5) | 296 (56.9) | |
| Ever | 242 (46.5) | 224 (43.1) | |
| Drinking status | | | 0.303 |
| Never | 395 (76.0) | 358 (68.8) | |
| Ever | 125 (24.0) | 162 (31.2) | |
| Stage | | | |
| Localized (I + II) | 408 (78.5) | | |
| Advanced (III + IV) | 112 (21.5) | | |
| Grade | | | |
| Well (I + II) | 385 (74.0) | | |
| Moderately (III) | 89 (17.1) | | |
| Poorly (IV) | 46 (8.9) | | |

Student's t-test for age and BMI distributions between cases and controls. Two-sided $\chi^2$ test for the frequency distribution of selected variables between renal cell carcinoma cases and cancer-free controls. SD, standard deviation; BMI, body mass index.

edge, no reports have been published regarding the role of the IL-8 polymorphism in RCC. The aim of our study was to investigate the possible association between the polymorphisms of IL-8-251 (rs4073) and +781 (rs2227306) and risk of RCC in Chinese Han population.

## Material & methods

### *Study population*

The case-control study included 520 histopathologically confirmed clear cell RCC patients and 520 cancer-free controls. Consecutive RCC patients were recruited between May 2004 and September 2010 at Tianjin Medical University General Hospital. The control group comprised 520 healthy volunteers for the general health checkup in our hospital during the same period. All the healthy controls had been under the health screening, and their clinical characteristics were matched to the sex and age distribution with the cases, as outlined in **Table 1**. After signed informed consent was obtained from all individuals, each subjects donated 5 ml blood used for genomic DNA extraction. Each participant was interviewed using a standard questionnaire by a trained nurse, to collect medical histories, demographic characteristics. The present study was performed with strict protocol under the Ethics Committee of Tianjin Medical University General Hospital. All the specimens we recruited were of Chinese Han ethnicity and were filtered based on their clinical characteristics. Before the assay, we obtained a written informed consent from each participant in our study.

### *DNA extraction and genotyping*

The polymorphisms in the promoters of the IL-8 genes analyzed in this study are shown in **Table 2**. The polymerase chain reaction (PCR) combined with the restriction fragment length polymorphism (RFLP) was used to determine the IL-8 genotypes. Genomic DNA was isolated from leukocytes of venous blood by proteinase K digestion and phenol/chloroform extraction. Genotyping of these six polymorphisms were all conducted with the MGB TaqMan Probe Assay (7900 HT Real-Time PCR System, Applied Biosystems, Foster City, CA). For quality control, genotyping was repeated randomly in at least 5% of the samples, and two of the authors independently reviewed all results. PCR reactions were carried out in a total volume 10 μl containing 20 ng of genomic DNA, 0.25 mM of each Dntp (Ecogen, Biologia Molecular S.L.), 0.2 units of Taq polymerase (Biotools, Inc.) and 2.5 pmol of each primer in a 1×PCR buffer (Sigma-Aldrich Co.). The details of the primers and PCR conditions used for the amplification of IL-8 are shown in **Table 2**.

### *Statistical analysis*

We used $\chi^2$ test (for categorical variables) or Student's t-test (for continuous variables) to evaluate the frequency distributions of selected demographic variables and each allele and genotype of SNPs between the cases and controls. Similarly, the Hardy-Weinberg equilibrium

**Table 2.** Details of PCR Primer sequences and RFLPs conditions in our study

| Gene | Polymorphism | SNP ID | Primer sequence | PCR Conditions |
|---|---|---|---|---|
| IL-8 | -251T/A | rs4073 | F: TCATCCATGATCTTGTTCTAA<br>R: GGAAAACGCTGTAGGTCAGA | 35 cycles: 95°C 40 s, 54°C 40 s, 72°C 60 s |
| IL-8 | +781C/T | rs2227306 | F: CTCTAACTCTTTATATAAGGAATT<br>R: GATTGATTTTATCAACAGGCA | 35 cycles: 94°C 180 s, 62°C 30 s, 72°C 30 s |

**Table 3.** Interaction analyses of the two SNP (-251T/A [rs4073], +781C/T [rs2227306]) of IL-8 gene polymorphisms to predict renal cell carcinoma risk

| Polymorphisms | Cases (N = 520) (%) | Controls (N = 520) (%) | OR (95% CI) | *P*-value* |
|---|---|---|---|---|
| -251T/A rs4073 | | | | |
| TT | 286 (55.0) | 328 (63.1) | 1 | |
| TA | 130 (25.0) | 104 (20.0) | 1.83 (1.23-3.95) | ***0.019**** |
| AA | 104 (20.0) | 88 (16.9) | 1.88 (1.29-3.68) | ***0.015**** |
| TA+AA | 234 (45.0) | 192 (36.9) | 1.85 (1.24-3.81) | ***0.018**** |
| T | 702 (67.5) | 760 (73.1) | 1 | |
| A | 338 (32.5) | 280 (26.9) | 1.92 (1.25-4.51) | ***0.013**** |
| +781C/T rs2227306 | | | | |
| CC | 237 (45.3) | 255 (47.4) | 1 | |
| CT | 213 (47.4) | 190 (46.3) | 1.30 (0.82-3.63) | 0.241 |
| TT | 70 (7.3) | 75 (6.3) | 1.45 (0.89-3.36) | 0.282 |
| CT+TT | 283 (54.7) | 265 (52.6) | 1.67 (0.87-3.41) | 0.169 |
| C | 687 (68.9) | 700 (70.5) | 1 | |
| T | 353 (31.1) | 340 (29.5 ) | 1.46 (1.19-.3.41) | 0.155 |

OR, odds ratio; CI, confidence interval. *Bold numbers indicate that the *P*-value is < 0.05.

(HWE) of each subject was examined by implying a two-sided chi-square ($\chi^2$) test which was performed by comparison of observed and expected genotype frequencies. The IL-8-251A/T and +781C/T polymorphisms genotypes related RCC risk was assessed by odds ratio (OR) and their corresponding respective confidence intervals 95% (CIs) value of the logistic regression, for both combined and respective genotype. We managed all the statistical analysis with the SPSS software version 19.0. A two-sided *P* value less than 0.05 was considered to be statistically significant for all the analyses.

## Results

*Characteristics of the study population*

This study included 520 RCC patients and 520 healthy controls, their age, gender, BMI, smoking status, drinking status, stage and grade were summarized in **Table 1**. The mean age (± SD) for case and control groups was 61.5 (13.3) and 59.7 (12.4) years, respectively. Our study included 520 RCC cases, including 367 males and 153 females, and 520 healthy controls, including 345 males and 175 females. No significant difference was detected in the age and gender distribution between two groups (P > 0.05). Regarding the clinical stage, 78.5% of patients were in stage I and II, and 21.5% were in stage III and IV. The control population was consistent with the Hardy-Weinberg Equilibrium (HWE) for the poly-morphisms in IL-8-251A/T and +781C/T.

*Distributions of IL-8-251A/T and +781C/T genotypes and risk of* RCC

The genotype and allele frequencies of the IL-8-251T/A (rs4073) and IL-8+781C/T (rs2227306) polymorphisms for all the studied variations are shown in **Table 3**. All genotype frequencies of the control group conformed to the Hardy-Weinberg equilibrium.

There were significant differences in the genotype and allele frequencies of IL-8-251T/A (rs4073) genotypes between RCC cases and controls. Compared with the IL-8 rs4073 homozygote TT, the heterozygous TA genotype was associated with significantly increased risk for RCC (OR = 1.83, 95% CI = (1.23-3.95), P = 0.019); the AA genotype was associated with increased risk for RCC (OR = 1.88, 95% CI = 1.29-3.68, P = 0.015). TA and AA combined variants were associated with increased risk for RCC compared with the TT genotype (OR = 1.85, 95% CI = 1.24-3.81, P = 0.018). However, the genotype and allele frequencies of IL-8 rs2227306 polymorphisms in RCC patients were not significantly different from controls (P > 0.05) as shown in **Table 3**.

**Table 4.** Association between IL-8 gene polymorphism (-251T/A, +781C/T) and clinicopathologic characteristics of renal cell carcinoma

| Genotypes Variable | n | -251T/A rs4073 | | | | +781C/T rs2227306 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | TT | TA | AA | *P* value | CC | CT | TT | *P* value |
| Age (years) | | 286 | 130 | 104 | | 237 | 213 | 70 | |
| ≤ 60 | 280 | 125 | 89 | 66 | 0.132 | 107 | 131 | 43 | 0. 231 |
| > 60 | 238 | 161 | 107 | 38 | | 130 | 115 | 27 | |
| Gender | | | | | | | | | |
| Male | 367 | 215 | 103 | 49 | 0.249 | 205 | 93 | 49 | 0. 408 |
| Female | 153 | 71 | 27 | 55 | | 81 | 32 | 25 | |
| Stage | | | | | | | | | |
| Localized (I + II) | 408 | 249 | 84 | 75 | 0.032* | 215 | 83 | 49 | 0.362 |
| Advanced (III + IV) | 112 | 37 | 46 | 29 | | 71 | 42 | 25 | |
| Grade | | | | | | | | | |
| Well (I + II) | 385 | 237 | 69 | 79 | 0.029* | 171 | 168 | 46 | 0.142 |
| Moderately (III) | 89 | 41 | 35 | 13 | | 41 | 32 | 16 | |
| Poorly (IV) | 46 | 8 | 26 | 12 | | 25 | 13 | 8 | |

*Student's t-test and the chi-square ($\chi^2$) test, $P < 0.05$.

*Distributions of IL-8-251A/T and +781C/T genotypes and clinicopathological characteristics*

The relationships between the IL-8-251A/T and +781C/T genotypes polymorphisms and clinicopathological parameters were calculated. The results are given in **Table 4**. For IL-8-251T/A rs4073, the genotype AA frequency in tumor metastasis patients was greater compared to patients without tumor metastasis, and the difference in frequency distribution between genotypes reached significance (P = 0.032). The similar result was fond with respect to grade No significant difference was observed with respect to age, gender and the IL-8 rs4073 genotypes. For IL-8+781C/T rs2227306, there are no any obvious differences in the relations between their age, gender, BMI, smoking status, drinking status, stage and grade respectively, and IL-8 rs2227306 genotypes.

## Discussion

In current hospital based case-control study, we assessed the association between the polymorphisms of two SNPs of IL-8 (rs4073-251T/A, rs2227306+781C/T) and risk of RCC in Chinese Han population and found the significant association between IL-8 rs4073-251T/A polymorphisms and risk of RCC. The genotype and allele distribution of polymorphisms rs4073-251T/A of IL-8 genotypes were significantly different between case and control groups, indicating that rs4073-251T/A of IL-8 might be related to RCC development.

Moreover, our results showed the genotype AA frequency of IL-8-251T/A rs4073 in tumor metastasis patients was greater compared to patients without tumor metastasis. These results indicated that the genotype AA of IL-8-251T/A carried a higher risk of RCC metastasis and later stages, compared with the TT genotype. To the best of our knowledge, our study is the first report to describe the possible role of two polymorphisms of IL-8 (rs4073 -251T/A, rs2227306 +781-C/T) as a risk factor for RCC and found that IL-8 rs4073 genotype variations do influence susceptibility to RCC development and metastasis in the Chinese Han population.

RCC is a common malignant tumor, which exists widely in the bone of children and adolescents [14]. It aroused people's concern universally owing to its highly malignant, facilely reversion, and readily metastases [15]. Up to now, inaugural mechanism of RCC was considered as a complex process and was not clear, but it was universally acknowledged that environment carcinogens could induce genomic polymorphism, such as oxidative stress, drinking, smoking, and ionizing radiation [16]. Interleukin-8, a member of the chemokine family, is produced by a wide range of normal cells including monocytes, neutrophils, fibroblasts, and endothelial cells, as well as by several types of tumor cells [17]. It was originally described as a chemoattractant for neutrophils and lymphocytes [18] and recently linked to cancer progression through its functions as mitogenic, motogenic, and angiogenic factor [13]. Recent studies revealed that IL-8 is overexpressed in a range of human cancers including nasopharyngeal [19], breast [20], and gastric cancers [21] and may, thus, constitute a risk factor in the development of solid tumors.

In agreement with our findings, several studies reported a relationship between the IL-8-251

(T/A) gene polymorphism and human cancer. This polymorphism is associated with a high risk of occurrence of gastric cancer, a strong neutrophil infiltration, an increased risk of lymph node metastasis and a poor differentiation of tumors [18]. The IL-8 (-251) A allele was also associated with a higher risk of prostate cancer [22], colorectal cancer [23], and oral squamous cell carcinoma [24]. There is now compelling evidence that these correlations are the result of increased levels of IL-8 protein, which may impact cancer development via regulation of immune responses and pathways of tumor angiogenesis and cancer progression [24]. IL-8 is also an important chemoattractant, involved in the activation and migration of lymphocytes and neutrophils into tissues and, thereby, is a major contributing factor involved in the initiation and amplification of the inflammatory response [18].

In spite of interesting findings on the association of IL-8 polymorphisms with RCC risk, there were several limitations that need to be addressed regarding the present study. We did not collect lifestyle data for individual participants, e.g. on local environmental factors, diet, or level of physical activity, which potentially could interact with genetic variations in influencing overall risk of developing RCC. Besides, the relative small sample size might hide some weak gene-disease association and gene-environment interactions. Studies need to be performed in larger study groups to confirm our preliminary results.

In conclusion, our study provided the evidence of association between the polymorphisms of IL-8-251 rs4073 and +781 rs2227306 and the risk of RCC and found the IL-8-251A/T genotype was associated with increased risk for development and metastasis of RCC in Chinese Han population. Because this is the first report concerning the IL-8 polymorphism and the risk of RCC in the literature, studies with larger sample size and further investigations into the mechanism are warranted to clarify and validate the role of IL-8 polymorphisms in RCC carcinogenesis.

## Disclosure of conflict of interest

None.

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