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Association between the interleukin-6 gene polymorphisms and renal cancer risk

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ABSTRACT

Introduction: Interleukin-6 (*IL-6*), a central proinflammatory cytokine, may be involved in the host response to cancer. We therefore aimed to evaluate the association of the *IL-6* gene polymorphisms at positions -174 and -572 with predisposition to renal cancer.
Materials and methods: We conducted a hospital-based case–control study. A total of 432 subjects, including 216 pathologically-proven renal cancer cases and 216 age- and gender-matched healthy controls, were recruited in this study. Polymorphism for the *IL-6* gene was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).
Results: Patients with renal cancer had a significantly higher frequency of *IL-6* -174 CC genotype [odds ratio (OR) = 2.08, 95% confidence interval (CI) = 1.05, 4.13; $P = 0.04$] than healthy controls. When stratifying by the grade, patients with higher grade (grade 3 or 4) renal cancer had a significantly higher frequency of *IL-6* -174 CC genotype (OR = 2.33, 95% CI = 1.04, 5.23; $P = 0.04$).
Conclusion: This study is, to our knowledge, the first to examine prospectively an increased risk role of *IL-6* -174 CC genotype in renal cancer susceptibility.

1. Introduction

Renal cancer is the predominant form of malignancy of the kidney (>80%) and represents 3–4% of all cancers [1]. About 63,920 new cases of renal cancer in the US are expected to be diagnosed in 2014, according to the American Cancer Society [1]. Epidemiological studies have shown that environmental factors, such as smoking, diesel exhaust, and various dioxins, may be involved in the development of sporadic renal cancer [2–4]. However, many people are exposed to these risk factors during their lifetime but only a fraction of them develop renal cancer, which suggests that genetic susceptibility may play a role in the etiology of this disease [5]. But the genetic basis of this cancer is not fully understood.

Interleukin-6 (*IL-6*), a phosphorylated glycoprotein containing 185 amino acids, is a multifunctional protein principally involved in different physiologic and pathophysiologic processes such as inflammation, bone metabolism, synthesis of C-reactive protein, and carcinogenesis [6,7]. The *IL-6* gene in humans is organized in five exons and four introns and maps to the short arm of chromosome 7 (7p21) [8]. The *IL-6* gene presents two biallelic polymorphisms at positions -174 and -572 in its promoter region [9,10]. It has been found that *IL-6* promoter polymorphisms (-174 G/C and -572 G/C) were associated with various cancers risks [11,12]. *IL-6* is a recently characterized pleiotropic cytokine with antitumor activity, including renal cancer [13,14].

We hypothesized that *IL-6* promoter polymorphisms (-174 G/C and -572 G/C) were associated with renal cancer risk. To test this hypothesis, we performed a prospective hospital-based case–control study to evaluate the association of the *IL-6* gene polymorphisms at positions -174 and -572 with predisposition to renal cancer.

2. Materials and methods

2.1. Study population

We conducted a hospital-based case–control study. A total of 432 subjects, including 216 renal cancer cases and 216 age- and gender-matched healthy controls, were recruited in this study between February 2012 and December 2014 in the West China

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Table 1
Restriction enzyme and primer sequences of *IL-6* SNPs.

SNP	Enzyme	Forward primer	Reverse primer	Product
-174 G/C	*Nla*III	5′-TTGTCAAGACATGCCAAGTGCT-3′	5′-GCCTCAGAGACATCTCCAGTCC-3′	GG: 13 bp, 227 bp, 59 bp; CC: 13 bp, 118 bp, 109 bp, 59 bp
-572 G/C	*Bsr*BI	5′-GGAGACGCCTTGAAGTAACTGC-3′	5′-GAGTTTCCTCTGACTCCATCGCAG-3′	CC: 163 bp; GG: 102 bp, 61 bp

Hospital of Sichuan University. All cases were histopathologically confirmed as renal cancer. For these cases, clinical and pathological information was extracted including tumor grade, tumor classification, lymph node invasion status, distant metastasis status and the pathology of renal cancer. To ascertain that volunteers were healthy and free of cancer, they all underwent various tests that included physical exams, questionnaires about their health and history, chest X-rays, blood and urine tests for various tumor markers, abdominal ultrasound, gastric endoscopy, and colon enema. All data points were collected through interviews with the patient or their families/surrogates. All parts of the study were approved by the Institutional Ethical Committee of the West China Hospital of Sichuan University, and informed consent according to the Declaration of Helsinki was obtained from all participants or their families/surrogates.

2.2. DNA extraction and genotyping

Genomic DNA was isolated from 20 g/L ethylenediaminetetraacetic acid (EDTA) or sodium citrate anticoagulated 3–5 ml venous blood by the commercially available Qiagen kit (QIAGEN Inc., Valencia, CA, USA) and stored at 4 °C. Polymorphism for the *IL-6* gene was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Restriction enzyme and primer sequences of *IL-6* promoter single nucleotide polymorphisms (SNPs) were listed in Table 1. PCR was performed in 25 μl using 100 ng genomic DNA as a template, 0.4 μM of each primer, 80 μM dNTP, and ~2 U DNA polymerase. PCR included 5-min denaturation at 95 °C and 35 amplification cycles (40 s at 95 °C, 30 s at 60 °C and 40 s at 72 °C for *IL-6* -572 and 1 min at 95 °C, 1 min at 53 °C, and 1 min at 72 °C for *IL-6* -174) and final elongation 5 min at 72 °C. Digestion was performed at 37 °C in 15 μl using 6–10 μl of PCR product and 3 U of restriction endonuclease for 2 h. Electrophoresis in a 2.5% agarose gel followed by ethidium bromide staining and ultraviolet illumination allowed detection of the alleles. For quality control, two independent observers, read all genotypes without knowing about the case or control status. When replicate quality control samples were evaluated, genotypes showed 100% concordance.

2.3. Statistical analysis

The Statistical Analysis System software (Version 9.1; SAS Institute Inc., Cary, NC, USA) was used for all statistical tests. Data are presented as mean ± standard deviation (SD) or as percentages for categorical variables. Comparisons between groups were made with χ^2 test (nominal data) or Student *t*-test (interval data). The existence of differences in genotypic frequencies between groups was assessed by means of Pearson χ^2 test and calculating the odds ratio (OR) with the 95% confidence intervals (CI). The *P*-value less than 0.05 was considered statistically significant.

Table 2
Distribution of characteristics of renal cancer cases and healthy controls.

	Cases	Controls	*P*
Number of subjects	216	216	
Sex (Male/Female)	165/135	163/137	0.87
Age (years), (mean ± SD)	43.6 ± 9.1	44.1 ± 9.3	0.51
Grade			
1	55 (25.5)		
2	129 (59.7)		
3 + 4	32 (14.8)		
Tumor classification			
T1	103 (47.7)		
T2	49 (22.7)		
T3	57 (26.4)		
T4	7 (3.2)		
Lymph node invasion status			
Negative	199 (92.1)		
Positive	17 (7.9)		
Distant metastasis status			
Negative	188 (87.0)		
Positive	28 (13.0)		
Pathology			
Clear cell carcinoma	197 (91.2)		
Granular cell carcinoma	15 (6.9)		
Chromophobe cell carcinoma	4 (1.9)		

Abbreviations: SD, standard deviation.

3. Results

3.1. Characteristics of participants

Characteristics of renal cancer cases and healthy controls were showed in Table 2. No significant differences were found between the renal cancer cases and healthy controls in sex or age. For the tumor grade, 55 (25.5%) were grade 1 renal cancer, 129 (59.7%) were grade 2 renal cancer, and 32 (14.8%) were higher grade (grade 3 or 4) renal cancer. For the tumor classification, 103 (47.7%) were T1 renal cancer, 49 (22.7%) were T2 renal cancer, 57 (26.4%) were T3 renal cancer, and 7 (3.2%) were T4 renal cancer. For the lymph node invasion status, 199 (92.1%) were negative renal cancer, and 17 (7.9%) were positive renal cancer. For the distant metastasis status, 188 (87.0%) were negative renal cancer, and 28 (13.0%) were positive renal cancer. For the pathology, 197 (91.2%) were clear cell

Table 3
Genotype and allele frequencies of *IL-6* gene polymorphisms among renal cancer cases and healthy controls.

Genotypes	Cases (%)	Controls (%)	OR (95%CI)	*P*
-174 GG	92 (42.6)	99 (45.8)	1.00 (Reference)	
-174 GC	95 (44.0)	102 (47.2)	1.00 (0.67, 1.49)	0.99
-174 CC	29 (13.4)	15 (6.9)	2.08 (1.05, 4.13)	0.04
-174 G allele frequency	279 (64.6)	300 (69.4)	1.00 (Reference)	
-174 C allele frequency	153 (35.4)	132 (30.6)	1.25 (0.94, 1.66)	0.13
-572 GG	126 (58.3)	131 (60.6)	1.00 (Reference)	
-572 GC	72 (33.3)	63 (29.2)	1.19 (0.78, 1.80)	0.42
-572 CC	18 (8.3)	22 (10.2)	0.85 (0.44, 1.66)	0.64
-572 G allele frequency	324 (75.0)	325 (75.2)	1.00 (Reference)	
-572 C allele frequency	108 (25.0)	107 (24.8)	1.01 (0.74, 1.38)	0.94