Germline Genetic Variations at 11q13 and 12p11 Locus Modulate Age at Onset for Renal Cell Carcinoma

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Purpose: Few risk factors have been identified for renal cell carcinoma. We performed a validation study in a population with a European background to identify the most significant variants previously identified in association with renal cell carcinoma risk.

Materials and Methods: We performed a case-control validation study after recruiting 463 controls and 463 patients with a histologically confirmed diagnosis of clear cell renal cell carcinoma. For each patient and matched control we genotyped 8 single nucleotide polymorphisms selected from previous studies to evaluate the association between candidate single nucleotide polymorphisms and renal cell carcinoma susceptibility.

Results: After adjusting for patient age, gender, smoking status and body mass index the AG + AA genotypes from rs7105934 (11q13) were associated with a decreased risk of renal cell carcinoma (OR 0.50, 95% CI 0.33–0.75, p = 0.001) and the AC + CC genotypes from rs1049380 (*ITPR2*) were associated with an increased risk (OR 1.66, 95% CI 1.28–2.16, p <0.001). Kidney cancer developed at an older age in patients carrying the dominant risk allele A for rs7105934 (mean age at diagnosis 73.1 vs 68.9 years, p = 0.002) and at a younger age in those carrying the dominant allele C for rs1049380 (mean 68.1 vs 70.8 years, p = 0.005).

Conclusions: In what is to our knowledge the first validation study of the main 8 single nucleotide polymorphism variants associated with renal cell carcinoma susceptibility we confirmed the association of 2 single nucleotide polymorphisms with the risk of renal cell carcinoma. Each variant influenced patient age at disease diagnosis.

Key Words: kidney; carcinoma, renal cell; risk; polymorphism, genetic; genotype

Abbreviations and Acronyms

AGT = angiotensinogen
BMI = body mass index
ccRCC = clear cell RCC
EPAS1 = endothelial PAS domain protein 1
GWAS = genome-wide association study
ITPR2 = inositol 1,4,5-triphosphate receptor, type 2
PCR = polymerase chain reaction
RCC = renal cell carcinoma
SNP = single nucleotide polymorphism

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KIDNEY cancer is the third most common urological malignancy after prostate and bladder cancer with approximately 209,000 new cases per year worldwide. However, it is the most lethal urological malignancy

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with 102,000 deaths per year.[1] RCC accounts for approximately 80% to 90% of all kidney cancer cases and ccRCC is the most common form of adult kidney cancer.

Sporadic RCC is a multifactorial disease. To date epidemiological studies have highlighted at least 4 risk factors for RCC, including hypertension, obesity, smoking and a first-degree relative with RCC.[2,3] Each of these factors results from the interaction of multiple genetic and environmental determinants.

Case-control association studies proved that SNPs are genetic variants that influence individual cancer susceptibility. GWASs have already identified more than 800 associations between genetic variants and approximately 150 common diseases, including prostate, bladder and kidney cancer.[4]

Using a candidate gene approach to clarify the role of hypertension in RCC Andreotti et al identified 2 SNPs (rs1326889 and rs2493137) located in the promoter of the *AGT* gene associated with RCC (table 1).[5] In 2010 Purdue et al reported the first GWAS of RCC, noting 6 SNPs at a significant level approaching or surpassing genome-wide significance.[6] However, only 2 of these loci on regions 2p21 (rs11894252 and rs7579899) and 11q13.3 (rs7105934) remained significantly associated with RCC susceptibility when the study was extended further. More recently, an independent GWAS identified a novel susceptibility locus on 12p11.23 in the *ITPR2* gene.[7]

We performed a validation study in a population with a European background by genotyping patients and controls for the 8 most significant variants identified in these previous studies (table 1). We also analyzed the association of these variants with susceptibility to RCC.

MATERIALS AND METHODS

Study Population

We performed a case-control validation study in 463 patients with a histologically confirmed diagnosis of ccRCC. All patients were recruited between 2002 and 2010 at the department of urology at Pitié-Salpêtrière Hospital, Tenon Hospital, Georges Pompidou European Hospital and Angers Hospital in France, where they were treated and followed for ccRCC. For each patient certain data were collected at the study inclusion visit, including age, personal and familial history of cancer, height, weight, BMI, self-reported hypertension and smoking status. Patients who had smoked at least 20 cigarettes per day for 5 years were defined as smokers. Smokers who had ceased smoking by the time of study inclusion were classified as former smokers. All study patients were white and treated with surgery. Those with a familial RCC syndrome, such as von Hippel-Lindau syndrome, were excluded from analysis. All surgical specimens were examined by dedicated genitourinary pathologists. Tumors were staged according to the 2009 UICC TNM classification.

We also included 463 benign controls in the study who presented to our hospital for the management of noncancerous urological disease, eg urolithiasis or benign prostatic hyperplasia, and who had no personal history of cancer. Controls were matched for gender and age more or less than 5 years.

We prospectively collected saliva from all patients and controls with the Oragene™ DNA collection kit. DNA extraction and frozen storage of all samples were done at a central laboratory according to a standardized protocol. All patients and controls provided written informed consent to participate in the study. The Assistance Publique-Hôpitaux de Paris ethics committee and the Comité de Protection des Personnes Ile de France IV institutional review board approved the study protocol.

Analysis

Genotyping. Eight SNPs selected from previous studies were tested. Genotyping was performed by the 5′ nuclease PCR method using TaqMan® assays. The final PCR reaction volume was 2 μl, composed of 5 ng DNA, 0.05 μl 40 × assay mix and 0.4 μl 5 × RealTime Ready™ DNA Probe Master Mix. After a first step at 95C for 1 minute the thermal cycling conditions were 45 cycles at 95C for 30 seconds and at 60C for 30 seconds. PCR and end point analysis were performed with the LightCycler® 1536 Real-Time PCR System.

Statistical. Study genotypes were tested for consistency with the expected genotypic frequencies under Hardy-Weinberg equilibrium in the control population. Genotypic frequency between cases and controls, and correlations with clinical and pathological data were assessed using chi-square analysis. The OR and 95% CI were calculated using logistic regression models with adjustment for gender, age, smoking status and BMI at interview. A case-control approach was achieved initially. All cases and controls were then collapsed into

Table 1. *Characteristics of most significant or promising SNPs associated with RCC susceptibility in previous studies*

References (locus/gene region)	SNP	OR (95% CI)	p Value	No. Cases/Controls
Andreotti et al[5] (1q42/*AGT*)	rs1326889, rs2493137	1.35 (1.15—1.58), 1.31 (1.12—1.54)	0.0002, 0.001	777/1,035
Purdue et al:[6]				5,970/13,423
2p21/*EPAS1*	rs7579899, rs11894252	1.15 (1.10—1.21), 1.14 (1.09—1.20)	2.3×10^{-9}, 1.8×10^{-8}	
3q26/*PLD1*	rs9839909	0.90 (0.86—0.95)	4.0×10^{-5}	
11q13/chr 11	rs7105934	0.69 (0.62—0.76)	7.8×10^{-14}	
12q24/*SCARB1*	rs4765623	1.15 (1.09—1.20)	2.6×10^{-8}	
Wu et al[7] (12p11/*ITPR2*)	rs1049380	1.18 (1.12—1.25)	6.07×1^{-9}	3,772/8,505