RESEARCH COMMUNICATION

Relationship Between CYP1A1 Genetic Polymorphisms and Renal Cancer in China

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Abstract

Aim: To study the potential role of cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) polymorphisms in the risk of renal cell cancer in Chinese. Methods: A total of 181 pathologically-proven renal cancers and 350 controls from the second Xiangya Hospital in Changsha were collected during the period from May 2007 to December 2010. CYP1A1 genetic polymorphisms were genotyped using PCR-RFLP. Unconditional logistic regression analysis was performed to analyze their relationship with risk of RCC. Results: Individuals with Val/Val genotypes had a significantly increased risk of RCC compared those with CYP1A1 IIe/IIe (OR=1.69, 95%CI=1.03-2.85). We also found CYP1A1 Wt/Vt and Vt/Vt to confer a significantly greater risk than CYP1A1 Wt/Wt (Wt/Vt: OR=2.14, 95%CI=1.24-3.45; Vt/Vt: OR=1.78, 95%CI=1.31-3.96). In smokers, a high increase risk of RCC was observed in those with CYP1A1 Val allele and Vt allele (Val allele: OR=2.13, 95%CI=1.40-2.57; Vt allele: OR=3.75, 95%CI=2.43-6.79), but no other significant interactions were found. Conclusion: Our study found suggestive evidence that CYP1A1 polymorphisms may play an important role in the etiology of RCC. Cigarette smoking may increase the susceptibility to RCC carcinogenesis in individuals with a high-risk genotype.

Key words: Renal cell cancer - CYP1A1 - genetic polymorphism - China

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Introduction

Renal cell cancer (RCC) is the most common renal tumor, and this type of cancer was estimated to be 6th most common malignancy worldwide in 2008. The number of new cases of renal tumor worldwide reached to 273,518 (IACR, 2008), and reported age-standardized incidence rates varied considerably by geographical region (Ferlay et al., 2004), with the highest incidence observed in Europe and northern America (Mathew et al., 2002). The incidence rates seem to be substantially lower among Asians, both in most Asian countries and the USA (Mathew et al., 2002; Hollingsworth et al., 2006). These observations provide that the differences in the prevalence may be related to the environment and genetic factors.

The causes of RCC have not been identified yet, but the evidence from clinical trials and medical experience built up over time reveals a strong connection between several lifestyle risk factors such as cigarette smoking, overweight, obesity, hypertension and antihypertensive drugs. Genetic and hereditary conditions such as Von Hippel-Lindau disease, tuberous sclerosis, hereditary papillary renal cell carcinoma, Birt-Hogg-Dube syndrome, hereditary leiomyomatosis renal cell carcinoma syndrome and polycystic kidney disease could increase the risk of RCC.

Current published evidence suggests that both environmental and genetic factors influence the pathogenesis of RCC (Weikert et al., 2010). Cytochrome P450, family 1, subfamily A, polypeptide 1(CYP1A1) is involved in xenobiotic metabolism and classified as a phase I enzyme. The expression of the CYP1A1 is induced in a ligand-dependent fashion by the aryl hydrocarbon receptor and arylhydrocarbon receptor nuclear translocator (Kawajiri, 1999; Hildebrandt et al., 2007). The CYP1A1 gene plays an important role in carcinogenesis of various cancers, and it might affect carcinogenesis of RCC through alteration of genotyoxicity. It is inhibited by fluoroquinolones and macrolides, induced by aromatic hydrocarbons. In addition, the CYP1A1 gene may convert environmental procarcinogens to reactive intermediates having carcinogenic effects (Greenlee et al., 2001). Two common polymorphisms in CYP1A1 have been reported: one is a T/C substitution located 264 bp

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downstream from the 3'-flanking region, forming an Msp1 restriction site (CYP1A1m1); the second is a G/A substitution at the 4889 bp position of exon 7, which leads to an amino acid substitution (Ile to Val) of its protein (CYP1A1m2) (Smith et al., 1998; Kawajiri, 1999). The association of these CYP1A1 single nucleotide polymorphisms (SNPs) with cancer (eg, lung, oesophageal, breast, oral cavity cancers) has been well documented. But there are few studies on the association between CYP1A1 and RCC up to now.

More recently, the association between CYP1A1 and RCC has been reported in east of China, but the sample of this study is small and only one common polymorphism in CYP1A1 was studied. Therefore, we conducted a case-control study to concerning the potential role of CYP1A1 polymorphisms in RCC susceptibility.

Materials and Methods

Patients and controls

Patients were selected from inpatients of the second Xiangya Hospital in Changsha during the period from May 2007 to December 2010. All cases recruited in this study were examined histologically confirmed. Among a total of 192 eligible cases, 181 were interviewed with a participation rate of 94.3%. Controls were randomly selected from people who requested general health examinations in the same hospital during the same period. Controls were randomly selected from people who requested general health examinations in the same hospital during the same period. Controls were required to be without any history of any type of cancer and frequency matched by five year age groups, with a control to case ratio of two, whenever possible. Among a total of 384 eligible controls, 350 were successfully interviewed with a participation rate of 91.2%.

Data collection

Trained interviewers conducted face-to-face interviews using a structured questionnaire to collect information on sociodemographic characteristics and potential confounding factors. Collected potential confounders mainly included tobacco smoking (Never, quite, current), alcohol use (Never, quite, current), antropometric measures (height and weight), family history, hypertension and previous diseases. Cancer patients were asked to refer about habits a year before the disease diagnosed.

Genotyping

Blood samples were from patients and controls and stored at -20°C. Genomic DNA were isolated from peripheral white blood cells using a Wizard Genomic DNA purification Kit. The genotyping of CYP1A1 MspI polymoprisim and Ile/Val polymorphism were determined by PCR-based RFLP methods. The genomic DNA was amplified by using the following primers: MspI polymorphism sense primer (50-CAGTGAAGAGGTGTAGCCGCT-30) and antisense primer (50-TAGGAGTCTTGTCTCATGCCT-30) and Ile/Val polymorphism sense primer (50-GAACTGCCACTTCAGCTGTCT-30) and antisense primer (50-GAAAGACCTCCCAGCGGTCA-30). These sets of primers were used for amplification of 100 ng of genomic DNA as a template. The PCR reaction volume was 50 ml and contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM dNTPs and 10 pmol each of the primers. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 65 s, at 60°C for 65 s, at 72°C for 90 s, and a final extension at 72°C for 5 min. After transient centrifugation, agarose electrophoresis was conducted.

Statistical analysis

Differences in the distributions of demographic characteristics, selected variables, and frequencies of genotypes of the CYP1A1 polymorphism between the cases and controls were evaluated by using the Student's t-test (for continuous variables) or chi-square test (for categorical variables). The associations between the CYP1A1 genotypes and risk of RCC were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from unconditional logistic regression analysis with the adjustment for possible confounders. Hardy–Weinberg equilibrium was tested using a goodness-of fit chi-square test. $p<0.05$ was considered statistically significant, and all statistical tests were two sided. All the statistical analyses were performed with the software Stata version 9 (Stata, College Station, TX).

Results

The characteristics of the 181 RCC cases and 350 controls enrolled in this study were shown in Table 1. There were no statistically significant differences between the cases and controls in terms of age, sex and drinking. But the cases had higher BMI than that of controls ($p<0.05$). There were more smokers among the cases (57%) than among the controls (39%, $p<0.001$).

The genotype distributions of CYP1A1 polymorphism in cases and controls were summarized in Table 2. The frequencies of CYP1A1 IIe/IIe, IIe/Val, and Val/Val in the RCC patients were 43%, 35% and 23%, respectively. The frequencies of the CYP1A1 gene IIe/Val polymorphism in controls were according to the Hardy-Weinberg equilibrium ($p=0.11$). Individuals with Val/Val genotypes had slight significant increased risk of RCC compared those with CYP1A1 IIe/IIe (OR=1.69, 95%CI=1.03-2.85). The frequencies of wild-type (Wt) and variant-type (Vt) MspI polymorphisms of CYP1A1 Wt/Wt, Wt/Vt and Vt/Vt in the RCC patients were

Table 1. Characteristics of RCC Cases and Controls

Variables		Cases	Controls	P value
Age[1]		53.6±10.2	52.7±11.3	0.18
Sex	Male	112 (62)	210 (60)	0.68
	Female	69 (38)	140 (40)	
BMI[2]		24.7±3.2	23.6±3.1	<0.001
First degree[3]		43 (24)	39 (11)	<0.001
Drinking	Never	69 (38)	157 (45)	0.14
	Ever	112 (62)	193 (55)	
Smoking	Never	77 (43)	212 (61)	<0.001
status	Ever	104 (57)	138 (39)	

[1]mean±SD years; [2]mean±SD kg/m^2; [3]relative with cancer

Table 2. Genotype Distributions of CYP1A1 Polymorphism in Cases and Controls

CYP1A1	Cases N=181(%)	Controls N=350(%)	OR[1]	P value
IIe/Val polymorphism				
IIe/IIe	77(43)	174(50)	1.0 (reference)	-
IIe/Val	63(35)	122(35)	1.20(0.69-1.81)	0.69
Val/Val	41(23)	54(15)	1.69(1.03-2.85)	0.026
IIe/Val+ Val/Val	104(57)	176(50)	1.21(0.87-1.84)	0.24
Msp I polymorphism				
Wt/Wt	80(44)	237(68)	1.0 (reference)	-
Wt/Vt	83(46)	94(27)	2.14(1.24-3.45)	<0.001
Vt/Vt	18(10)	19(5)	1.78(1.31-3.96)	0.005
Wt/Vt+ Vt/Vt	101(56)	135(39)	1.95 (1.61-3.53)	<0.001

[1]Adjusted for age, sex, BMI, first degree relative with cancer and smoking.

Table 3. Association Between the CYP1A1 Polymorphisms and Smoking on RCC

Smoking status			Genotypes	
	Cases/Controls	OR*	Cases/Controls	OR*
IIe/Val polymorphism				
	IIe/IIe		IIe/Val+ Val/Val	
	77/174		104/176	
Never	52/136	1.0(reference)	25/76	0.93(0.57-1.62)
Ever	25/38	1.72(0.90-3.25)	79/100	2.13(1.40-2.57)
Msp I polymorphism				
	Wt/Wt		Wt/Vt+ Vt/Vt	
	80/237		101/135	
Never	35/135	1.0(reference)	42/77	1.87(1.03-3.76)
Ever	45/102	1.21(0.66-2.09)	59/58	3.75(2.43-6.79)

*95% CI

44%, 46% and 10%, respectively. The frequencies of MspI polymorphisms in controls were also according to the Hardy-Weinberg equilibrium (p=0.07). Individuals carrying CYP1A1 Wt/Vt and Vt/Vt showed a significant increased risk of RCC compared those with CYP1A1 Wt/Wt (Wt/Vt: OR=2.14, 95%CI=1.24-3.45; Vt/Vt: OR=1.78, 95%CI=1.31-3.96). Further, a slight significant increased risk of RCC was found in those carrying CYP1A1 Vt allele compared those with CYP1A1 Wt/Wt (OR=1.95, 95%CI=1.61-3.53).

The impact of combination of CYP1A1 polymorphisms and smoking on RCC was showed in Table 3. Among the smokers, individuals with CYP1A1 Val allele and Vt allele showed a significant high increased risk of RCC (Val allele: OR=2.13, 95%CI=1.40-2.57; Vt allele: OR=3.75, 95%CI=2.43-6.79). But no significant interaction was found between smoking and CYP1A1 genotypes (p=0.13).

Discussion

The present study conducted in a Chinese population investigated the CYP1A1 polymorphism on RCC risk. This study suggested that Wt/Vt and Vt/Vt genotype was significant associated with RCC risk. Such findings were in line with those of a previous study in east China that the OR was 2.5 for those with Wt/Vt genotype (Wang et al., 2008). However, another study conducted in Netherlands did not show significant increased risk (Smits et al., 2008). The inconsistency of these studies may be explained by differences in population background, study design, sample size, and also by change. Further confirmation of existing findings is still needed in future studies.

CYP1A1 polymorphism is likely to play an important role in the etiology of cancer through its function in activating environmental procarcinogens and alteration of genotyoxicity. MspI polymorphism of CYP1A1 was found to be associated with lung cancer, prostate cancer and colorectal cancer (Nakachi et al., 1993; Sivaraman et al., 1994; Kawajiri et al., 1996; Shaik et al., 2009). IIe/Val polymorphism was reported to be associated with lung cancer (Hayashi et al., 1991; Sivaraman et al., 1994) and squamous cell carcinoma of oral cancer (Zhuo et al., 2009). The noncoding region is important for mRNA stability and expression of gene products. CYP1A1 gene expression is modulated by post-transcription and post-translation mechanisms (Kruys et al., 1987; Levy et al., 1995; Tanguay et al., 1996). MspI polymorphism in the noncoding 3'-region seems to control CYP1A1 gene expression. IIe/Val polymorphism in the hemeblinding region modulates the enzyme activity of CYP1A1 (Kawajiri et al., 1993). It has It has been reported that steroid hydroxylation activities of CYP1A1 variant enzymes are different (Schwarz et al., 2000). CYP1A1 variant enzymes can increase these enzyme activities. Our study showed Val/Val was associated with increased risk of RCC, this suggested the hypothesis that CYP1A1 polymorphism play a role in carcinogenesis of RCC.

In the present study, we evaluated the interaction between CYP1A1 genotypes and smoking in patients with RCC. Our study showed smokers had higher RCC risk than non-smokers. This implied that polymorphisms in metabolic genes might greatly increase susceptibility carcinogens to RCC in smokers, possibly by interfering with the detoxification of carcinogens present in cigarette smoke. However, we did not find the significant interaction between them. This implied that in patients with a high-risk genotype, smoking was involved in tumor promotion rather than tumor initiation (Smits et al., 2008). Previous study also

showed that cigarette smoking played a role of RCC promotion, which is in line with our study.

This study has several major strengthens. First, an extensive effort was made to collect information on major risks for RCC, which was further considered and adjusted throughout the analysis. Second, in many hospital-based studies, controls were taken from hospitalized individuals with higher chance than the general population to share a common exposure with cases, which may be a threat to the validity of the results. In this study controls were selected from those who came to hospitals for routine health examination, probably making the controls more representative of the general population. However, since it was a non-randomized sampling in our study, there was still a certain risk of selection bias if people who seek routine health examination may be more likely to pay attention to their health.

In conclusion, this study found suggestive evidence that CYP1A1 polymorphism may play an important role in the etiology of RCC. Cigarette smoking may increase the susceptibility carcinogenesis of individual with high-risk genotype to RCC. Further studies in Chinese population with larger sample size are still warranted.

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