RESEARCH COMMUNICATION

Interleukin-4-Receptor Alpha Gene Polymorphism and the Risk of Renal Cell Carcinoma in a South Indian Population

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Abstract

The renal cell carcinoma (RCC) is a rare condition, accounting for only 3% of all adult malignancies although constituting 90% of kidney cancers. The tumor is immunogenic and the host immune system may modulate the clinical course of the disease. It has been reported that genetic polymorphisms in the interleukin-4-receptor alpha gene are associated with risk and prognosis in RCCs. The present study is aimed at analyzing the presence and significance of the interleukin-4-receptor alpha *Ile50Val* and *Gln576Arg* polymorphisms in a group of RCC patients from South India. PCR-RFLP analysis was performed on genomic DNA isolated from blood samples and the genotypes deduced. A significant association was found between the IL4 R alpha *Val/Val* genotype and increased risk of RCC (OR: 3.45, CI: 1.15–10.38, P: 0.04). The *Val/Val* genotype was also found to be significantly associated with increased risk in individuals below 54 years (OR: 5.79, CI: 1.33–25.07 P: 0.03) of age and in females (OR: 7.47, CI: 1.4–39.84, P: 0.03). However, no significant association was observed with the *Gln576Arg* polymorphism. Stratified analysis based on the genotypes and the stage of tumor revealed no significant association. Thus, the present study indicates that IL4Ralpha could be a candidate gene for assessing the risk of RCC.

Key Words: Renal cell carcinoma - IL4Ralpha gene polymorphism - South India

Asian Pacific J Cancer Prev, **10**, 295-298

Introduction

Renal cell carcinoma (RCC) is the most common malignancy of the kidney, the frequency of which is increasing in both, men and women. Renal Cell carcinoma has a worldwide incidence. Number of deaths worldwide from kidney cancer exceeded 100,000 in 2001 (Sachdeva, 2008). RCC develops in males almost twice as often as in females and shows a peak in the sixth decade of life (Godley and Stinchcombe, 1999). There is no clear geographical or ethnic preference. An increased incidence of RCC has been associated with end-stage renal disease and with acquired cystic kidney disease. RCCs are often large at detection and frequently already have metastasized (van den Berg and Storkel, 2003).

The exact causes of renal cell carcinoma have not been identified yet, but the evidence from clinical trials and medical experience built up over time reveals a strong connection between several risk factors such as gender, age and lifestyle. It is reported that it is more common in men than women (Woldrich et al., 2008). Smoking, alcohol intake and obesity are among other lifestyle factors that are known to increase the risk of RCC. Genetic and hereditary conditions such as Von Hippel-Lindau (VHL) disease, tuberous sclerosis, hereditary papillary renal cell carcinoma (HPRCC), Birt-Hogg-Dube syndrome, hereditary leiomyomatosis renal cell carcinoma syndrome and polycystic kidney disease also increase the risk of RCC. Certain treatments such as dialysis or medications (including certain pain relievers such as Phenacetin) have also been reported to increase the risk of renal cell carcinoma.

IL4R is a heterodimer comprising the IL4Rα and γc chains. The IL4-receptor represents a complex transmembrane receptor composed of at least two different proteins, the common γ chain shared by several interleukin receptors and a 140 kDa high affinity binding chain (IL4Rα) (Deichmann et al., 1997). Both chains are members of the hematopoietin receptor superfamily. The alpha-chain binds IL4 and mediates its effect through kinases of the Janus family attached to the intracellular domain. The coding gene has been localized to the short arm of chromosome 16 (16p12.1). Many studies have reported polymorphic sites resulting in the substitution of Ile for Val (Ile50Val) and Arg for Gln (Gln576Arg) in the extracellular and cytoplasmic domains respectively (Noguchi et al., 1999a & 1999b). It is believed that both, the Ile and Arg alleles, which strengthen signals through the IL4R, predispose the host immune system to Th2 cytokines.

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Materials and Methods

Subjects

The present case control study comprised 50 RCC patients and 51 unrelated healthy control subjects. Table 1 lists the characteristics of the study subjects. 45 patients had undergone surgical resection of the primary tumor, three of them had lung metastasis and one had lung, liver and bone metastasis. All the individuals included in the study were of South Indian ethnicity. The age of patients ranged from 40 to 85 years with a mean age of 54. Control subjects were in the age group of 40 to 70 years with a mean age of 51 years. Pathological grading of the tumors and tumor stage information were obtained. 24 patients had stage I, 12 had stage II, 12 had stage III and two had stage IV RCC. All subjects were enrolled after obtaining informed consent. The study was approved by the Medical Ethics Committee of the institution.

Genotyping of IL4R alpha polymorphisms

Blood samples (5ml) were collected from both, the cases and control subjects, in EDTA vials. Genomic DNA was isolated from the blood samples by salting out procedure (Maniatis et al., 1982). The primer pairs used for the analysis of both SNPs were described previously (Noguchi et al., 1999a & 1999b). The *Ile50Val* polymorphism was genotyped by a mismatch PCR-RFLP method. The 273-bp fragment in which the *RSa*I restriction site was introduced by a single base pair mismatched antisense primer was amplified using primer pairs GGCAGGTGTGAGGAGCATCC and GCCTCCGTTGTTCTCAGGTA.

The *Gln576Arg* polymorphism was also genotyped by mismatch PCR-RFLP method. A single base pair mismatch was introduced in the sense primer to obtain the *MSp*I restriction site. Primer pairs were CCCCCACCAGTGGCTACC and GCCCCAAACCCACATTTC. The cycling conditions were 94º C for 5min for one cycle; 94º C for 45sec, 60º C for 45sec and 72º C for 1 min for 30 cycles; and a final elongation cycle of 72ºC for 5 min. The PCR products were visualized by 3.5% agarose gel electrophoresis and the genotypes determined by the restriction pattern. For the *Ile50Val* polymorphism, the *Ile/Ile* genotype resulted in an undigested band of 273 bp, the Val allele resulted in two fragments of 254 bp and 19 bp each, and an *Ile/Val* genotype was characterized by three fragments of 273bp, 254 bp and 19 bp each. For the *Gln576Arg* polymorphism, the *Gln/Gln* resulted in an undigested 163 bp product, the *Arg/Arg* genotype with a 145 bp product and an 18bp product and the *Gln/Arg* genotype with a 163 bp, a 145 bp and an 18bp product.

Statistical analysis

The allele frequency and genotype frequency of *Ile50Val* and *Gln576Arg* were calculated for cases and controls. The expected genotype frequencies were calculated for the patients and controls to test if the population followed Hardy-Weinberg equilibrium. The association between IL4R (*Ile50Val* and *Gln576Arg*) polymorphisms and the risk of renal cell carcinoma was determined by calculating the odds ratio (OR) at 95% confidence intervals (CI) for each polymorphism. OR at 95% confidence interval was also calculated for both the polymorphisms combined. The cancer risk was also analyzed by stratifying the subjects based on mean age, gender and tumor stage and calculating OR at 95% CI. P value of <0.05 was considered statistically significant. For cell values of 0, 0.5 was added to all the cells in the table following which OR at 95% CI were obtained (Mehta et al., 1985). All statistical analyses were done by using SPSS statistical package (Version 15).

Table 1. Characteristics of the Study Subjects

Variable		Controls (51)	Cases (50)
Gender	Male	21	37
	Female	30	13
Age	Mean	51	58
	Minimum	40	24
	Maximum	70	84
Tumor Stage	Stage I	NA	24
	Stage II	NA	12
	Stage III	NA	12
	Stage IV	NA	2

NA- Not Applicable

Results

The allele and genotype frequencies of *Ile/Val* and *Gln/Arg* polymorphisms are shown in Tables 2 and 3. The control group followed Hardy-Weinberg equilibrium for both the polymorphisms whereas the cases group did not

Table 2. Association between IL4R alpha *Ile50Val* and *Gln576Arg* Polymorphisms and Renal Cell Carcinomas - Alleles

Variable	Genotype frequency			N	Alleles		P value
	Ile/Ile	*Ile/Val*	*Val/Val*		w	v	
Ile / Val	CONTROLS						
Observed	23	22	6	51	0.67	0.33	0.978
Expected	22.7	22.7	5.7				
	CASES						
Observed	20	12	18	50	0.52	0.48	0.001
Expected	13.5	25.0	11.5				
	Gln/Gln	*Gln/Arg*	*Arg/Arg*				
Gln / Arg	CONTROLS						
Observed	31	17	3	51	0.77	0.23	0.948
Expected	30.6	17.8	2.6				
	CASES						
Observed	37	9	4	50	0.83	0.17	0.038
Expected	34.4	14.1	1.4				

Table 3. Association between IL4R alpha *Ile50Val* and *Gln576Arg* Polymorphisms and Renal Cell Carcinomas - OR* Values

Genotype		Genotype frequency		OR (95% CI)	P-value
		Controls	Cases		
Ile / Val	*Ile/Ile*	23	20	1	
	Ile/Val	22	12	0.63 (0.25-1.58)	NS
	Val/Val	6	18	3.45 (1.15-10.4)	0.04
Gln / Arg	*Gln/Gln*	31	37	1	
	Gln/Arg	17	9	0.44 (0.17-1.13)	NS
	Arg/Arg	3	4	1.12 (0.23-5.38)	NS

*Odds Ratios (95% Confidence Limits); NS, Not significant

Table 4. Association between IL4R alpha Polymorphisms (*Ile50Val* and *Gln576Arg*) and Renal Cell Carcinomas Based on Age at Diagnosis

Genotype		Genotype frequency Controls	Cases	OR (95% CI)	P-value
Ile / Val					
≤ 54	*Ile/Ile*	18	7	1	
	Ile/Val	14	1	0.18 (0.02-1.67)	NS
	Val/Val	4	9	5.79 (1.33-25.1)	0.03
> 54	*Ile/Ile*	5	13	1	
	Ile/Val	8	11	0.53 (0.13-2.09)	NS
	Val/Val	2	9	1.73 (0.27-11.0)	NS
Gln / Arg					
≤ 54	*Gln/Gln*	20	13	1	
	Gln/Arg	13	4	0.47 (0.13-1.77)	NS
	Arg/Arg	3	0	0.22 (0.01-4.54)	NS
> 54	*Gln/Gln*	11	24	1	
	Gln/Arg	4	5	0.57 (0.13-2.56)	NS
	Arg/Arg	0	4	4.22 (0.21-85.2)	NS

NS- Not Significant

Table 5. Association between IL4Rα Polymorphisms (*Ile50Val* and *Gln576Arg*) and Renal Cell Carcinomas Based on Gender

Genotype		Genotype frequency Controls	Cases	OR (95% CI)	P-value
Ile / Val					
Male	*Ile/Ile*	9	15	1	
	Ile/Val	9	12	0.80 (0.24-2.65)	NS
	Val/Val	3	10	2.00 (0.43-9.26)	NS
Ile / Val					
Female	*Ile/Ile*	14	5	1	
	Ile/Val	13	0	0.10 (0.01-1.94)	NS
	Val/Val	3	8	7.47 (1.40-39.8)	0.03
Gln / Arg					
Male	*Gln/Gln*	15	27	1	
	Gln/Arg	5	6	0.67 (0.17-2.56)	NS
	Arg/Arg	1	4	2.22 (0.23-21.7)	NS
Gln / Arg					
Female	*Gln/Gln*	16	10	1	
	Gln/Arg	12	3	0.40 (0.09-1.78)	NS
	Arg/Arg	2	0	0.31 (0.01-7.21)	NS

NS- Not Significant

(Table 2). With respect to the *Ile/Val* polymorphism, the frequency of the Val allele was 0.48 in cases, which was higher than controls, with a frequency of 0.33. Of the 51 controls analyzed, 45.1% were homozygous wild type (Ile/Ile), 43.1 % were heterozygous (*Ile/Val*) and 11.8% were *Val/Val* homozygous variant. In the 50 cases, 40% had *Ile/Ile*, 24% had *Ile/Val* and 36% had *Val/Val* genotype. Thus, the frequency of *Val/Val* genotype was significantly higher in cases than the controls suggesting the association of IL4R-alpha *Val/Val* genotype with increased risk of renal cell carcinoma (OR: 3.45, 95% CI: 1.15-10.38, P: 0.04). The frequency distribution of *Gln/Arg* was not significantly different between the cases and controls for both, heterozygous and variant genotypes (Table 3).

To evaluate the interaction between the genotypes, we examined the combined effect of the *Ile/Val* and *Gln/Arg* genotypes. Taking the risk of the combined wild type genotypes as a baseline reference category, the odds ratios were calculated for the combination of the *Ile/Val* and *Gln/Arg* genotypes. The analysis revealed no statistically significant difference between cases and controls when the variant genotypes occurred in combination.

To study the association between IL4Rα polymorphisms (*Ile50Val* and *Gln576Arg*) and renal cell carcinoma based on age at diagnosis, the study subjects were classified into two groups based on their age at diagnosis of RCC. The two groups were ≤ 54 and > 54. The risk associated with IL4Rα genotypes was calculated in each group. In the ≤ 54 group comprising controls, *Val/Val* was observed in 11.1%, *Ile/Val* was observed in 38.9% and *Ile/Ile* was observed in 50% of the subjects. In the cases group, *Val/Val* was observed in 52.9%, *Ile/Val* was observed in 5.9% and *Ile/Ile* was observed in 41.2% of the subjects. With *Ile/Ile* as reference, the odds ratio was calculated to be 0.1837 (0.02-1.67) for *Ile/Val* and 5.79 (1.34-25.07) for *Val/Val*. A significant association (P: 0.03) was found between the subjects of the age group ≤54 and IL4Rα polymorphism (*Ile50Val*). No significant association was found between the subjects of the age group > 54 and IL4Rα polymorphism (*Ile50Val*). Also, no significant association was found between the subjects of both age groups and IL4Rα *Gln576Arg* polymorphism (Table 4). Further analysis based on the gender revealed no significant association for both the polymorphisms in males, however, the *Val/Val* genotype was associated with increased risk in females (P: 0.03, OR: 7.47, CI: 1.4-39.84) (Table 5). Stratified analysis based on the stage of tumor revealed no significant association for both the polymorphisms.

Discussion

In the present study, two genetic polymorphisms in the Interleukin-4-receptor alpha gene have been investigated and their association with risk and prognosis in renal cell carcinoma evaluated. Renal cell carcinoma is a malignancy of complex etiology. A number of risk factors are identified of which genetic polymorphisms are known to play a significant role. The tumor is immunogenic and responds best to immunotherapy. The cytokine pathways in RCC are not clearly known yet and hence, an understanding of the role of respective genes could prove useful for the same purpose.

The two polymorphic sites analyzed in this study were reported to be associated with risk and prognosis in RCC in Japanese population. So far, this association has not been reported in other populations. The role of IL4Rα signaling pathway in RCC is not clear, however, it presents the possibility that the dominant genetic effects of polymorphisms on increasing the risk of RCC are attributable to the direct effects of signaling through the receptor.

The present study shows that the *Val/Val* genotype was found to be significantly associated with increased risk (OR: 3.45, CI: 1.15-10.38, P: 0.04) for renal cell carcinoma. The other genotypes, however, were not statistically significant in association with the risk. This finding deviates from that reported for Japanese.

In the analysis of association of the genotypes with age of subjects in the present study, it was found that

subjects below the age of 54 who had the *Val/Val* genotype (OR: 5.79, CI: 1.33–25.07, P: 0.03) were at increased risk than others. No significant association was found between the other genotypes and age of the subjects. No such associations with age were reported in the Japanese study. The present study failed to show a significant association between stage of the tumor and the gene polymorphisms though there was a two-fold greater association in cases with higher stage tumors and *Ile/Val* and *Val/Val* genotypes. However, Nakamura et al (2002) reported that *Ile/Ile* genotype showed a trend toward exhibiting higher tumor stages and unfavorable prognosis than the *Ile/Val* or *Val/Val*, not in concordance with the present results.

Analyzing male and female subjects for their risk to develop RCC, a significant association (OR: 7.47, CI: 1.4–39.84, P: 0.03) was found between female subjects possessing the *Val/Val* allele and increased risk of RCC. This association was not investigated in the Japanese population. In general, it is reported that men are twice as likely to develop RCC, and the risk allele demonstrated has been Ile. However, the finding in the present study is in contrast to what has been reported.

The presence of the two polymorphisms has been identified in RCC patients in this study. However, it is not clear whether one or both polymorphic sites are in association with risk of RCC. Linkage studies performed by $\chi 2$ test to determine the existence of linkage disequilibrium between the two polymorphic sites reveal the presence of LD. This finding correlates with previous reports. The study in Japanese population showed the possibility of the linkage disequilibrium causing a positive association of both polymorphisms with increased risk. In addition, the possibility that neither of these polymorphic sites are actually involved directly and that they could be in linkage with another important RCC susceptibility gene cannot be excluded.

Genetic polymorphisms vary in their frequency from one population to another. Also, within a population, there is a possibility for genetic heterogeneity to play a role. Studies at a larger scale will have to be performed in various populations, in order to understand the role of any polymorphism completely. The present study intended to be an elementary step in that direction.

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