GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association between SNPs in vascular endothelial growth factor polymorphisms and risk of renal cell carcinoma: a case-control study

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Genet. Mol. Res. 14 (3): 11119-11125 (2015)
Received January 21, 2015
Accepted June 17, 2015
Published September 22, 2015
DOI http://dx.doi.org/10.4238/2015.September.22.5

ABSTRACT. We conducted this case-control study to assess the role of the VEGF -2578C/A, +1612G/A, +936C/T and -634G/C gene polymorphisms in the development of renal cell carcinoma (RCC). A hospital-based case-control study was conducted in a 360 consecutive primary RCC patients and 360 age and gender-matched controls during January 2010 and January 2014. The polymerase chain reaction-restriction fragment length polymorphism was used for VEGF -2578C/A, +1612G/A, +936C/T and -634G/C genotyping. Multivariate conditional logistic regression analyses showed that subjects carrying the AA and the CA+AA genotypes of VEGF -2578C/A had significant association with increased risk of RCC compared to those having the CC genotype, and the ORs (95%CI) were 1.77 (1.10-2.85) and 1.37 (1.01-1.86), respectively. Using the conditional logistic regression model, CA+AA genotype of VEGF -2578C/A had a significantly increased risk of RCC in ever cigarette smokers, and individuals with hypertension, and the ORs (95%CI) were 1.93 (1.08-3.45) and

2.57 (1.06-6.57), respectively. In conclusion, our results showed that AA genotype of VEGF -2578C/A genetic variants is associated with increased risk of RCC.

Key words: Vascular endothelial growth factor; Polymorphism; Renal cell carcinoma; Cancer risk

INTRODUCTION

In genitourinary cancers, renal cell carcinoma (RCC) has the highest mortality rate. It is a heterogeneous malignancy, and also revealed molecular and genetic heterogeneity and complexity (Tomaszewski et al., 2014). Clear cell renal cell carcinoma is the most popular histological subtype, accounting for approximately 80% of the cases of renal tumors (Farhadi et al., 2014). Early diagnosis and medical treatment seems important in decreasing mortality and increasing total quality of life. Several studies have suggested that genetic factors are involved in the development of RCC (Wang et al., 2014; Meng et al., 2014, 2015).

Angiogenesis is the formation of new blood vessels from pre-existing endothelium, and it is a discrete event in carcinogenesis which is correlated with the aggressive potential of a tumor (Hanahan and Folkman, 1996; Nakamura, et al., 2005). Increasing evidences have indicated that the growth of tumors is associated with increased angiogenesis, and formation of new blood vessels is a fundamental step in tumor development and expansion (Mariani et al., 2012). Vascular endothelial growth factor (VEGF) is a critical angiogenesis promoter, which is encoded by the VEGF gene (Hicklin and Ellis, 2005). It is reported that several common VEGF gene polymorphisms are associated with the development and progression of solid tumors, including 1154G/A (rs1570360), 2634G/C (rs2010963), and 2460C/T (rs833061) (Sun et al., 2013; Xu and Zhu, 2014; Jannuzzi et al., 2015).

Only three previous studies have reported the association between SNPs in VEGF and risk of RCC (Abe et al., 2002; Ajaz et al., 2011; Sáenz-López et al., 2013), but the findings of these studies are inconclusive. One recent meta-analysis have reported no correlation between six common SNPs in the VEGF gene and the development of RCC due to limited published studies (Zhang et al., 2013). Therefore, we conducted this case-control study to assess the role of the -2578C/A, +1612G/A, +936C/T and -634G/C gene polymorphisms in the development of RCC.

MATERIAL AND METHODS

Study population

A hospital-based case-control study was conducted in a 360 consecutive primary RCC patients and 360 age and gender-matched controls during January 2010 and January 2014 in the Zhengzhou People's Hospital. All RCC patients were newly diagnosed and histopathologically confirmed independently by two gynecologic pathologists. The criteria for RCC cases were those who had not yet received any chemotherapy or radiotherapy.

In total, 360 control subjects were randomly selected from individuals in the routine health examination center during the same period in Zhengzhou People's Hospital. All control subjects were found to be free of cancer. Each control was matched with a case by gender and

age at enrollment (within ±5 years). All control subjects were confirmed to be lack of osteosarcoma, no history of any cancer, no family history of osteosarcoma in first degree relatives and a matched gender and age distribution with cases.

A written informed consent was obtained from each subject. The protocol of this study was previously approved by the ethics committee of Zhengzhou People's Hospital.

DNA extraction and genotype analysis

Each subject participant donated a 5-mL venous blood sample for genomic DNA extraction, which the blood was placed in 0.5 mg/mL EDTA anticoagulantcontained 0.5 mg/mL EDTA as anticoagulant, and the blood was stored atkept in -20ºC until usefurther usage. Genomic DNA was isolated from peripheral blood lymphocytes using Qiagen blood mini kit (Qiagen, Germany) by the manufacturer's protocol. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for VEGF -2578C/A, +1612G/A, +936C/T and -634G/C genotyping. The positive and reverse primers of these four were designed using the Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). Forward and reverse primers were respectively as follows: for -2578C/A, 5'-TACTGGGGAGGTAACCTA GCAC-3' and 5'-GGAAAAATTCCTGGCTGGTT-3'; for +1612G/A, 5'-CACATGCTGCACG CGCATCTCA-3' and 5'-ACCCCAGGAAGGGGAGCAGGA-3'; for +936C/T, 5'-AGGGTTC GGGAACCAGATC-3' and 5'-CTCGGTGATTTAGCAGCAAG-3'; and for -634G/C, 5'-GAGAGAAGTCGAGGAAGAGAGA-3' and 5'-CCCAAAAGCAGGTCACTCACTT-3'. For PCR amplification, the standard program was used as follows: predenaturing (94°C for 5 min); denaturing (94°C for 30 s);annealing (55°C for 30 s); lengthening (72°C for 45 s); total 35 cycles.

Statistical analysis

Continuous variables are reported as means ± SE, while categorical variables were shown as frequencies and percentages (%). Differences in the distributions of demographic and clinical characteristics between cases and controls were comapred by the χ^2-test. Deviations from Hardy-Weinberg equilibrium of the genotyped VEGF -2578C/A, +1612G/A, +936C/T and -634G/C genetic polymorphisms were evaluated by χ^2-test. Multivariate conditional logistic regression analyses were used for evaluating the association between VEGF -2578C/A, +1612G/A, +936C/T, and -634G/C genetic polymorphisms and RCC risk with adjustment for potential confounding factors, and the assessed results were calculated with odds ratio (OR) and 95% confidence intervals (CI). Gene-environmental interaction was evaluated by conditional logistic regression. A two-tailed P value of <0.05 was considered to be statistically significant. All the statistical analyses were conducted with the SPSS 19.0 statistical software (SPSS, Chicago, IL, USA).

RESULTS

The demographic and clinical characteristics of the study subjects are shown in Table 1. As expected, no significant differences were observed between the groups in terms of age and gender ($P > 0.05$). Compared with the control subjects, the RCC patients were more likely

to have a smoking habit and to suffer from diabetes. Of the 360 RCC patients, 303 (84.17%) patients had I-II stage of RCC, 217 (60.28%) had I-II grade of RCC, and 204 (82.50%) were clear-cell RCC.

Table 1. Demographic and clinical characteristics of all the RCC cancer cases and controls.

Characteristics	Cases	%	Controls	%	χ^2	P value
Age, years (mean ± SD)	52.7 ± 9.7		52.1 ± 9.8			
<55	199	55.28	192	53.33		
≥55	161	44.72	168	46.67	0.27	0.61
Gender						
Male	236	65.56	236	65.56		
Female	124	34.44	124	34.44	0.00	1.00
Cigarette smoking						
Never	229	63.61	256	71.11		
Ever	131	36.39	104	28.89	4.61	0.03
Alcohol drinking						
Never	253	70.28	268	74.44		
Ever	107	29.72	92	25.56	1.56	0.21
Hypertension						
No	233	64.72	257	71.39		
Yes	127	35.28	103	28.61	3.68	0.06
Diabetes						
No	296	82.22	328	91.11		
Yes	64	17.78	32	8.89	12.31	<0.001
Family history of cancer						
Never	330	91.67	341	94.72		
Ever	30	8.33	19	5.28	2.65	0.10
Stage						
I-II	303	84.17				
III-IV	57	15.83				
Grade						
I-II	217	60.28				
III-IV	143	39.72				
Histology						
Clear cell	297	82.50				
Papillary	16	4.44				
Chromophobe	24	6.67				
Others	23	6.39				

By χ^2-test, genotype frequencies of VEGF -2578C/A, +1612G/A, and -634G/C in controls demonstrated Hardy-Weinberg equilibrium, while +936C/T did not (Table 2). Multivariate conditional logistic regression analyses showed that subjects carrying the AA and the CA+AA genotypes of -2578C/A had significant association with increased risk of RCC compared to those having the CC genotype, and the ORs (95%CI) were 1.77 (1.10-2.85) and 1.37(1.01-1.86) for the AA and the CA+AA genotypes, respectively. However, we did not find significant association between VEGF +1612G/A, +936C/T and -634G/C polymorphisms and risk of RCC ($P > 0.05$).

In addition, we assessed the association between the VEGF -2578C/A polymorphism and demographic characteristics of RCC patients, including gender, age, cigarette smoking, alcohol drinking, hypertension, diabetes and family history of cancer (Table 3). Compared with CC genotype, CA+AA genotype of VEGF -2578C/A had a significantly increased risk of RCC in ever cigarette smokers, and individuals with hypertension, and the ORs (95%CI) were 1.93 (1.08-3.45) and 2.57 (1.06-6.57) for ever cigarette smokers and individuals with hypertension, respectively.

Table 2. Genotype frequencies of VEGF -2578C/A, +1612G/A, +936C/T, and -634G/C polymorphisms in RCC cases and controls.

VEGF genotypes	Cases	%	Controls	%	P value for Hardy-Weinberg equilibrium	Adjusted OR (95%CI)[1]	P value
-2578C/A							
CC	150	41.67	178	49.44		1.0 (Ref.)	-
CA	149	41.39	141	39.17		1.25 (0.90-1.74)	0.16
AA	61	16.94	41	11.39	0.11	1.77 (1.10-2.85)	0.01
CA+AA	210	58.33	182	50.56		1.37 (1.01-1.86)	0.04
+1612G/A							
GG	152	42.22	166	46.11		1.0 (Ref.)	-
CA	170	47.22	164	45.56		1.13 (0.82-1.56)	0.43
AA	39	10.83	30	8.33	0.23	1.42 (0.81-2.49)	0.19
CA+AA	209	58.06	194	53.89		1.18 (0.87-1.60)	0.28
+936C/T							
CC	224	62.22	240	66.67		1.0 (Ref.)	-
CT	81	22.50	73	20.28		1.19 (0.81-1.74)	0.35
TT	55	15.28	46	12.78	<0.001	1.28 (0.81-2.02)	0.26
CT+TT	136	37.78	119	33.06		1.22 (0.89-1.68)	0.19
-634G/C							
GG	121	33.61	134	37.22		1.0 (Ref.)	-
GC	170	47.22	163	45.28		1.15 (0.82-1.62)	0.39
CC	69	19.17	63	17.50	0.27	1.21 (0.78-1.89)	0.37
GC+CC	239	66.39	226	62.78		1.17 (0.85-1.61)	0.31

[1]Adjusted for gender, age, cigarette smoking, alcohol drinking, hypertension, diabetes, and family history of cancer in conditional logistic regression model.

Table 3. Stratification analyses between VEGF -2578C/A polymorphisms and risk of RCC.

Variables	Cases	Controls	Cases		Controls		Adjusted OR (95%CI)[1]	P value
			CC (N = 150)	CA+AA (N = 210)	CC (N = 178)	CA+AA (N = 182)	CA+AA *vs* CC	
Age, years								
<55	199	192	88	111	121	115	1.33 (0.89-1.97)	0.14
≥55	161	168	62	99	57	67	1.36 (0.82-2.25)	0.21
Gender								
Male	236	236	92	144	118	138	1.34 (0.92-1.95)	0.11
Female	124	124	58	66	60	44	1.55 (0.89-2.72)	0.1
Cigarette smoking								
Never	229	256	89	141	120	148	1.28 (0.88-1.87)	0.17
Ever	131	104	62	70	58	34	1.93 (1.08-3.45)	0.0003
Alcohol drinking								
Never	253	268	110	143	130	127	1.33 (0.92-1.91)	0.11
Ever	107	92	40	67	48	55	1.46 (0.81-2.63)	0.18
Hypertension								
No	233	257	92	142	156	172	1.40 (0.98-2.0)	0.06
Yes	127	103	59	69	22	10	2.57 (1.06-6.57)	0.02
Diabetes								
No	296	328	125	171	168	173	1.32 (0.96-1.84)	0.08
Yes	64	32	25	39	10	9	1.73 (0.54-5.55)	0.29
Family history of cancer								
Never	330	341	139	191	168	173	1.33 (0.97-1.83)	0.06
Ever	30	19	11	19	10	9	1.92 (0.51-7.21)	0.27

[1]Adjusted for gender, age, cigarette smoking, alcohol drinking, hypertension, diabetes, and family history of cancer in conditional logistic regression model.

DISCUSSION

In the present study, we investigated the influence of VEGF -2578C/A, +1612G/A,

+936C/T and -634G/C in the VEGF gene on the risk of RCC, and examined the effect of gene-environmental interaction on the development of this cancer in a Chinese population.

It is reported that angiogenesis is associated with the development of many tumors, and the VEGF is a potent regulator of angiogenesis and is involved in the carcinogenesis of solid tumors (Roy et al., 2006; Kushner and Bautch, 2013) The expression of VEGF could promote endothelial cell proliferation and remodel the extracellular matrix in the blood vessels (Ferrara, 2002), and functional gene variations of the VEGF gene could influence the gene expression and the plasma VEGF levels, and thus the accelerated process of carcinogenesis (Ajaz et al., 2011; Sáenz-López et al., 2013; Zhang et al., 2013).

Previous studies reported the association between polymorphisms in VEGF -2578C/A and risk of cancer (Tie et al., 2014; Zidi et al., 2014; Machado et al., 2014; Deng et al., 2014; Chen et al., 2014). A case-control study in a Chinese population suggested that VEGF -2578C/A polymorphism plays an important role in the pathogenesis of osteosarcoma (Tie et al., 2014). Another case-control study investigated the association between common SNPs in VEGF and risk of cervical cancer in a Tunisian population, and it suggested that VEGF -2578C/A polymorphism may contribute to the development of cervical cancer (Zidi et al., 2014). In a case-control study in a Portuguese population, findings suggested that VEGF -2578C/A polymorphism was associated with an increased risk of hepatocellular carcinoma (Machado et al., 2014). In a case-control study in a Chinese population, it reported an association between VEGF -2578AA genotypes and increased risk of lung cancer (Deng et al., 2014). However, some studies reported inconsistent results (Sa-Nguanraksa et al., 2013). In a case-control study in a Thai population, it did not find significant association between VEGF -2578C/A polymorphism and breast cancer risk (Sa-Nguanraksa et al., 2013; Sáenz-López et al., 2013). In a meta-analysis regarding the association between VEGF -2578C/A and cancer risk, this study indicates that VEGF -2578C/A polymorphism was only associated with the risk of colorectal cancer and lung cancer (Chen et al., 2014). The discrepancies between previous epidemiological studies could be due to differences in populations, source of included cases and controls, sample size, study design and also by chance.

For the association between VEGF -2578C/A polymorphism and risk of RCC, two previous studies reported their association (Ajaz et al., 2011; Sáenz-López et al., 2013). Ajaz et al. (2011) reported that VEGF -2578 A-allele and A-carrier genotypes were correlated with increased risk of RCC in a Pakistani population. However, another case-control study in a Spanish population suggested that VEGF -2578C/A polymorphism does not appear to exert a significant risk of RCC (Sáenz-López et al., 2013). In a recent meta-analysis, Zhang et al. reported that VEGF gene polymorphisms may be not associated with an increased risk of RCC (Zhang et al., 2013). Further large sample studies are greatly needed to confirm our finding.

In conclusion, our results showed that AA genotype of VEGF -2578C/A genetic variants is associated with increased risk of RCC, and a significant interaction was found between VEGF -2578C/A polymorphism and smoking and hypertension. Further investigations on the role of VEGF gene polymorphisms on the risk of RCC are greatly needed.

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