Single Nucleotide Polymorphisms in the 3' Untranslated Region of Vascular Endothelial Growth Factor Gene in Japanese Population with or without Renal Cell Carcinoma

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ABE, A., SATO, K., HABUCHI, T., WANG, L., LI, Z., TSUCHIYA, N., OHYAMA, C., SATOH, S., OGAWA, O. and KATO, T. *Single Nucleotide Polymorphisms in the 3' Untranslated Region of Vascular Endothelial Growth Factor Gene in Japanese Population with or without Renal Cell Carcinoma.* Tohoku J. Exp. Med., 2002, 198 (3), 181-190 — We investigated native Japanese subjects whether *C702T*, *C936T* and *G1612A* polymorphisms in the 3' untranslated region (3'-UTR) of vascular endothelial growth factor (VEGF) gene are associated with the risk of renal cell carcinoma (RCC). Genomic DNAs from 145 RCC patients and 145 healthy controls were examined by polymerase chain reaction-based restriction fragment length polymorphism. Variant allele frequencies of *C702T*, *C936T* and *G1612A* were 0.00, 0.20 and 0.13 in the controls, respectively. The *C702T* and *G1612A* allele frequencies were significantly different between the Japanese population and the Caucasian population reported elsewhere. For each of *C936T* and *G1612A* polymorphisms, there was no statistically significant difference in the distribution of genotype frequencies between the cases and controls. Odds ratios and 95% confidence intervals computed by logistic regression analyses were not statistically significant. Stratification for the RCC cases according to pathological cell subtype, grade or stage failed to reveal any significant heterogeneity with respect to the genotype of each VEGF polymorphism. We revealed that there are significant ethnic differences in the *C702T* and *G1612A* allele frequencies, but suggested that *C702T*, *C936T* and *G1612A* polymorphisms in the 3'-UTR of VEGF gene are not associated with the risk of RCC, at least in Japanese population. ——— vascular endothelial growth factor; polymorphisms; renal cell carcinoma; 3'untranslated region

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Renal cell carcinoma (RCC) is a representative malignant tumor of the kidney (Novick and Campbell 2002). The histological and cytological classification distinguishes clear cell carcinoma, chromophobe renal cell carcinoma and papillary renal cell carcinoma. Of these, the clear cell carcinoma is the most common type of RCC, and have a highly angiogenic feature with a high potential of intra-vascular spread to the distant organs. Previous studies by us and others have indicated that the microvessel density of RCC well correlates with the expression of vascular endothelial growth factor (VEGF) gene and its receptor genes (Sato et al. 1994; Takahashi et al. 1994; Sasaki 1996; Tomisawa et al. 1999; Slaton et al. 2001), and reflects the outcome of RCC patients (Jacobsen et al. 2000). The VEGF level in the peripheral blood circulation of RCC patients has also been mentioned to be significantly elevated as compared with that of normal subjects and correlated with the tumor size (Sato et al. 1999). These documents have suggested that angiogenic factors including VEGF may play a crucial role in the development or progression of RCC (Folkman 1992; Jacobsen et al. 2000; Slaton et al. 2001).

Among the various angiogenic factors, VEGF is considered to exert a most potent activity for angiogenesis and has been extensively investigated. VEGF is a transmembrane glycoprotein that interacts with specific receptors to regulate angiogenesis. Various genetic and epigenetic events are involved in the up-regulation of VEGF gene (Ferrara 2001). A recent study revealed that the *CC* genotype of *C936T* polymorphism in the 3' untranslated region (3'-UTR) of VEGF gene was associated with an increase in the VEGF level in the peripheral blood circulation as compared with the *CT* and *TT* genotypes (Renner et al. 2000).

In addition to the *C936T* polymorphism, two other single nucleotide polymorphisms, *C702T* and *G1612A*, have been identified in the 3'-UTR (Renner et al. 2000). Because RCC is

one of the highly vascularized solid tumors and associated with an increase in the VEGF level in the peripheral blood circulation (Sato et al. 1999; Jacobsen et al. 2000), the VEGF polymorphisms could be responsible for the individual susceptibility to development and progression of this tumor. Hence, we carried out this study to investigate on the native Japanese subjects whether these three polymorphisms of in the 3'-UTR of the VEGF gene are associated with the risk of development or progression of RCC.

MATERIAL AND METHODS

Subjects

One hundred forty-five patients with RCC treated at the Akita University Medical Center and the related hospitals were enrolled in the present study (Table 1). They were recruited for the study with a written informed consent. All subjects were diagnosed histologically on the specimens obtained from surgical resection. Clinical and histopathological information was obtained from the patient charts, imaging studies and pathological reports (Table 1). The information was reviewed and the data were entered into the database. Tumor stage was assigned according to the tumor-node-metastasis (TNM) staging system (Hermanek and Sobin 1992). Pathological grading of the RCC was determined according to the General Rule for Clinical and Pathological Studies on RCC by the Japanese Urological Association and the Japanese Society of Pathology (1999), which is based on the World Health Organization (WHO) criteria (Mostofi and Davis 1998). One hundred forty-five healthy native Japanese men and women in the Akita prefecture, who visited the community hospitals for a routine health checkup, were recruited for the study as controls with a written informed consent (Table 1). Our study was conducted as a part of an ongoing project to investigate the genetic susceptibility for RCC onset and progression, which was approved by the Internal Ethical Board of the Akita University School of

TABLE 1. *Demographic characteristics of renal cell carcinoma cases and healthy controls*

Variable	Cases (n=145) Number (%)	Controls (n=145) Number (%)
Age (years) (mean±s.d.)	60.4±11.1	50.3±8.0
< 62 ^a	70 (48.2)	133 (91.8)
≥ 62	75 (51.8)	12 (8.2)
Gender		
Male	108 (74.5)	108 (74.5)
Female	37 (25.5)	37 (25.5)
Tumor stage ^b		
I	82 (56.6)	
II	21 (14.5)	
III	19 (13.1)	
IV	23 (15.9)	
Tumor size (mm) (mean±s.d.)	52.7±30.1	
Histology		
Clear cell	140 (96.6)	
Others ^c	5 (3.4)	
Grade of tumor		
G1	83 (57.2)	
G2	42 (29.0)	
G3	17 (11.7)	

^aMedian age of the case group.^bI: T1, II: T2, III: T1-T3, N1 IV: T4+N2+M1.^cOthers include 1 chromophobe cell carcinoma and 4 papillary renal cell carcinomas.

Medicine.

Genotype of the VEGF polymorphism

DNA was extracted from blood samples collected from the patients with RCC and the control subjects using a QIAamp Blood Kit (QIAGEN, Hilden, Germany) or by the standard method with proteinase K digestion followed by phenol/chloroform extraction.

The 238-bp fragment of *C702T* polymorphism was amplified using specific primers: sense 5'-TCCATTTCCCTCAGATGTGACAAGCC-3' and antisense 5'-AGTCTCGCCCTCCGGACCCAAA-3' (Table 2). Polymerase chain reaction (PCR) was carried out in a 20 µl volume containing approximately 20 ng of genomic DNA, 1×PCR buffer supplied by a

manufacturer, 0.2 mM of each dNTP (dATP, dCTP, dGTP and dTTP), 1.8 mM MgCl₂, 30 pmol of each primer and 1.0 U of Ampli-Taq Gold DNA polymerase (Perkin-Elmer, Branchburg, NJ, USA). After the 10-minute initial denaturation step at 95°C, 35 cycles of PCR reaction consisting of 95°C for 20 seconds, 65°C for 30 seconds and 72°C for 30 seconds were carried out, followed by a 1-minute final extension step at 72°C in the thermal cycler (GeneAmp PCR System 9700: Perkin-Elmer). After confirmation of successful PCR amplification by 2.5% agarose gel electrophoresis, each PCR product was digested overnight with 5 U Msp I enzyme at 37°C (New England Biolabs Inc., Beverly, MA, USA) and was electrophoresed on 3.0% agarose gel. Restriction

TABLE 2. Three types of VEGF polymorphism and the primers of PCR-RFLP

DNA alteration	Restriction enzyme	Product length		Primer pair
		Wild type	Variant	
<i>C702T</i>	Msp I	188,40,14	226,14	P1:F:5'-TCCATTTCCCTCAGATGTGACAAGCC-3' P1:R:5'-AGTCTCGCCCTCCGGACCCAAA-3'
<i>C936T</i>	Nla III	126,86	208	P2:F:5'-AAGGAAGAGGAGACTCTGCGCAGAGC-3' P2:R:5'-TAAATGTATGTATGTGGGTGGGTGTGTCTACAGG-3'
<i>G1612A</i>	Mnl I	112,105	217	P3:F:5'-CACATGCTGCACGCGCATCTC-3' P3:R:5'-ACCCCAGGAAGGGGAGCAGGA-3'

VEGF, vascular endothelial growth factor; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

tion fragments were 226 bp and 14 bp for the *C* allele, and 188 bp, 40 bp and 14 bp for the *T* allele, respectively.

The 208-bp fragment of *C936T* polymorphism was amplified using specific primers: sense 5'-AAGGAAGAGGAGACTCTGCGCAGAGC-3' and antisense 5'-TAAATGTATGTATGTGGGTGGGTGTGTCTACAGG-3' (Table 2). PCRs were carried out in the same condition as for the *C702T* polymorphism. Each PCR product was digested overnight with 5 U Nla III enzyme at 37°C (New England Biolabs Inc.) and was electrophoresed on 3.0% agarose gel. Restriction fragments were 208 bp for the *C* allele, and 126 bp and 86 bp for the *T* allele, respectively.

The 217-bp fragment of *G1612A* polymorphism was amplified using specific primers: sense 5'-CACATGCTGCACGCGCATCTC-3' and antisense 5'-ACCCCAGGAAGGGGAGCAGGA-3' (Table 2). Each PCR product amplified in the same condition described above was digested overnight with 5 U Mnl I enzyme at 37°C (New England Biolabs Inc.) and was electrophoresed on 3.5% agarose gel. Restriction fragments were 217 bp for the *G* allele, and 112 bp and 105 bp for the *A* allele, respectively (Table 2).

Statistical analysis

All data were entered into a database and analyzed using Excel 2001 (Microsoft

Corporation, Tokyo) and SPSS (version 10.0J, SPSS Japan Inc., Tokyo) software. Hardy-Weinberg equilibrium analyses were performed to compare the observed and expected genotype frequencies using a chi-square test. Case-control data were analyzed by 2-sided 2×3 or 3×3 contingency tables according to the genotype using the Pearson chi-square test. The odds ratio (OR) and 95% confidence interval (95% CI) of VEGF genotype were calculated using the multivariate logistic regression model. In analyzing the relationship between the genotype and the disease status of RCC, the tumor stages were transformed to binary data (Stage I+II vs. Stage III+IV), the histological findings were transformed to binary data (clear cell type vs. the others including 1 chromophobe cell carcinoma and 4 papillary renal cell carcinomas), and the tumor grade were transformed to binary data (grade 1 vs. grade 2+3). A multivariate logistic regression analysis was performed after adjusted for age and gender. All statistical tests and *p*-values were 2-tailed and a result was considered significant when a *p*-value was less than 0.05.

RESULTS

Subject characteristics

Brief clinical and pathological characteristics of the subjects in this study are presented in Table 1. The case group was comprised of 108 males and 37 females, and the control group was

comprised of 108 males and 37 females. The mean age $\pm$ s.d. was 60.4 ± 11.1 years in the case group and 50.3 ± 8.0 years in the control group, respectively.

VEGF polymorphism in RCC and control subjects

We found no polymorphism in *C702T* in both of the case group and the control group. Thus, the subsequent analyses were performed on the other two polymorphisms. The frequencies of the other VEGF genotypes of *C936T* and *G1612A* in the case group and the control group are shown in Table 3. The genotype distribution in both groups was in the Hardy-Weinberg equilibrium ($df=1$, $p>0.05$, data not shown).

There was no significant difference in the calculated allelic frequencies of the *C936T* polymorphism between the case group (*C* allele, 0.81; *T* allele, 0.19; $p=0.75$) and the control group (*C* allele, 0.80; *T* allele, 0.20). Also there was no significant difference in the calculated allelic frequencies of the *G1612A* polymorphism between the case group (*G* allele, 0.89; *A* allele, 0.11; $p=0.45$) and the control group (*G*

allele, 0.87; *A* allele, 0.13).

Statistical analysis of the genotype prevalence for the *C936T* polymorphism showed that no significant differences were found between the case group (*CC* 0.67, *CT* 0.28 and *TT* 0.05; $p=0.21$) and the control group (*CC* 0.62, *CT* 0.36 and *TT* 0.02). The *G1612A* polymorphism also showed no significant differences between the case group (*GG* 0.78, *GA* 0.21 and *AA* 0.01; $p=0.57$) and the control group (*GG* 0.75, *GA* 0.23 and *AA* 0.02).

VEGF polymorphism and tumor status

The RCC group was classified into the low stage (stages I+II) and the high stage (stages III+IV), and the genotype frequencies of each polymorphism in both groups were compared (Tables 4 and 5). The frequencies of the *CC* and *CT+TT* genotypes for the *C936T* polymorphism were 0.68 and 0.32 in the low-stage group, and 0.64 and 0.35 in the high-stage group, respectively. There was no significant difference in these genotype frequencies between the low- and high-stage tumors ($p=0.67$). For the *G1642A* polymorphism, the frequencies of the *GG* and *GA+AA* genotypes were 0.75 and

TABLE 3. *VEGF* genotype frequencies and odds ratios

Genotype	Cases (%) <i>n</i> = 145	Controls (%) <i>n</i> = 145	OR (95% CI)	aOR (95% CI)	<i>p</i>
<i>C936T</i>					
<i>CC</i>	97 (66.9)	90 (62.1)	1.00 (ref)	1.00 (ref)	0.206 ^a
<i>CT</i>	41 (28.3)	52 (35.9)	0.73 (0.54–8.63)	0.90 (0.52–1.59)	
<i>TT</i>	7 (4.8)	3 (2.1)	2.17 (0.44–1.21)	2.69 (0.57–12.8)	
<i>CT+TT</i>	48 (33.1)	55 (37.9)	0.81 (0.50–1.31)	0.99 (0.58–1.72)	
<i>G1612A</i>					
<i>GG</i>	113 (77.9)	109 (75.2)	1.00 (ref)	1.00 (ref)	0.567 ^b
<i>GA</i>	31 (21.4)	33 (22.8)	0.91 (0.52–1.58)	0.94 (0.51–1.75)	
<i>AA</i>	1 (0.7)	3 (2.1)	0.32 (0.03–3.14)	0.27 (0.02–3.99)	
<i>GA+AA</i>	32 (22.1)	36 (24.8)	0.86 (0.50–1.48)	0.89 (0.49–1.64)	

^aCases vs. controls.

^bCases vs. controls.

VEGF, vascular endothelial growth factor; OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio; ref, reference.

TABLE 4. *Characteristics of tumors and C936T VEGF genotypes in 145 RCC cases*

Variable	Genotype	OR (95% CI)		aOR (95% CI)	<i>p</i>
Stage		I+II	III+IV		
	<i>CC</i>	70 (68.0)	27 (64.3)	1.00 (ref)	1.00 (ref)
	<i>CT</i>	27 (26.2)	14 (33.3)	0.74 (0.34–1.63)	0.72 (0.32–1.61)
	<i>TT</i>	6 (5.8)	1 (2.4)	2.31 (0.27–20.13)	1.98 (0.22–17.73)
	<i>CT+TT</i>	33 (32.0)	15 (35.7)	0.85 (0.40–1.81)	0.81 (0.37–1.74)
Histology		Clear cell	Others		
	<i>CC</i>	84 (67.1)	3 (60.0)	—	—
	<i>CT</i>	39 (27.9)	2 (40.0)	—	—
	<i>TT</i>	7 (5.0)	0 (0.0)	—	—
	<i>CT+TT</i>	46 (32.9)	2 (40.0)	—	—
Grade		G1	G2+3		
	<i>CC</i>	58 (69.9)	37 (62.7)	1.00 (ref)	1.00 (ref)
	<i>CT</i>	21 (25.3)	19 (32.2)	1.42 (0.67–2.99)	1.67 (0.77–3.62)
	<i>TT</i>	4 (4.8)	3 (5.1)	1.18 (0.25–5.55)	1.35 (0.28–6.55)
	<i>CT+TT</i>	25 (30.1)	22 (37.3)	1.38 (0.68–2.79)	1.61 (0.77–3.36)

^aI+II vs. III+IV.^bI+II vs. III+IV.

VEGF, vascular endothelial growth factor; RCC, renal cell carcinoma; OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio; ref, reference.

TABLE 5. *Characteristics of tumors and G1612A VEGF genotypes in 145 RCC cases*

Variable	Genotype	OR (95% CI)		aOR (95% CI)	<i>p</i>
Stage		I+II	III+IV		
	<i>GG</i>	36 (85.7)	77 (74.8)	1.00 (ref)	1.00 (ref)
	<i>GA</i>	6 (14.3)	25 (24.3)	2.20 (0.82–5.96)	1.95 (0.74–5.16)
	<i>AA</i>	0 (0.0)	1 (1.0)	—	—
	<i>GA+AA</i>	6 (14.3)	26 (25.2)	2.03 (0.77–5.36)	2.00 (0.75–5.29)
Histology		Clear cell	Others		
	<i>GG</i>	110 (78.6)	3 (60.0)	—	—
	<i>GA</i>	29 (20.7)	2 (40.0)	—	—
	<i>AA</i>	1 (0.7)	0 (0.0)	—	—
	<i>GA+AA</i>	30 (21.4)	2 (40.0)	—	—
Grade		G1	G2+3		
	<i>GG</i>	64 (77.1)	47 (79.7)	1.00 (ref)	1.00 (ref)
	<i>GA</i>	19 (22.9)	11 (18.6)	0.97 (0.51–1.83)	0.79 (0.34–1.81)
	<i>AA</i>	0 (0.0)	1 (1.7)	—	—
	<i>GA+AA</i>	19 (22.9)	12 (20.3)	0.86 (0.38–1.94)	0.91 (0.40–2.07)

VEGF, vascular endothelial growth factor; RCC, renal cell carcinoma; OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio; ref, reference.

0.25 in the low-stage group, and 0.86 and 0.14 in the high-stage group, respectively. There was no significant difference in these genotype fre-

quencies between the two groups ($p=0.67$ for *C936T* and $p=0.15$ for *G1612A*).

As shown in Tables 4 and 5, we failed to

find any significant difference in genotype frequencies for *C936T* and *G1612A*.

We classified the RCC group into the low-grade (grade 1) and the high-grade (grade 2+3) groups according to the histological cell differentiation, and the genotype frequency of each polymorphism in both groups was compared (Tables 4 and 5). In each polymorphism, there was no significant difference in the genotype frequencies between the two groups ($p=0.37$ for *C936T* and $p=0.72$ for *G1612A*).

DISCUSSION

Renner et al. (2000) assessed the VEGF polymorphisms in the 3'-UTR among a healthy Caucasian population as shown in Table 6 and mentioned that the variant allele frequency of *C702T*, *C936T* and *G1612A* was 0.02, 0.16 and 0.47, respectively. The variant allele frequency of *C936T* and *G1612A* in a Japanese population was reported to be 0.15 and 0.13 by Awata et al. (2002), but they did not analyze the *C702T* polymorphism (Table 6). The present study showed that the variant allele frequency of *C702T*, *C936T* and *G1612A* in the

normal Japanese population was 0.00, 0.20 and 0.13, respectively. The results of the allele and genotype distribution for *C936T* by us were well consistent with those by Renner et al. (2000) and Awata et al. (2002), indicating no ethnic difference in this genotype between native Japanese and Caucasian. On the other hand, the data for the *G1612A* by us were well consistent with those by Awata et al. (2002), but showed a significant difference in the allele frequencies and genotype distributions as compared with those by Renner et al. (2000) ($p<0.001$; Table 6). In addition, our present study failed to detect the polymorphism of the *C702T* genotype in the native Japanese. These findings will indicate that there are considerable ethnic difference in the *G1612A* and *C702T* genotypes between Japanese and Caucasian.

It has been reported that 3'-UTR of VEGF gene acts as increasing the stability of mRNA and is associated with the hypoxic induction of VEGF (Dibbens et al. 1999; Awata et al. 2002; Greener et al. 2002; Iida et al. 2002). However, the mechanism for increasing the stability

TABLE 6. Polymorphisms in 3'-UTR of VEGF gene

Genotype	Controls (%) Present study <i>n</i> = 145	Controls (%) Awata et al. <i>n</i> = 118	Caucasian (%) Renner et al. <i>n</i> = 119	<i>p</i> *
<i>C702T</i>				
CC	145 (100)		115 (96.6)	
CT	0 (0)	—	4 (3.4)	—
TT	0 (0)		0 (0)	
<i>C936T</i>				
CC	90 (62.1)	85 (72.0)	85 (71.4)	
CT	52 (35.9)	31 (26.3)	30 (25.2)	0.160
TT	3 (2.1)	2 (1.7)	4 (3.4)	
<i>G1612A</i>				
GG	109 (75.2)	92 (78.0)	39 (32.8)	
GA	33 (22.8)	22 (18.6)	48 (40.3)	<0.001
AA	3 (2.1)	4 (3.4)	32 (26.9)	

*Present study vs. Renner et al. (2001), by Pearson chi-square test
UTR, untranslated region; VEGF, vascular endothelial growth factor.
Awata et al. (2002).

of the mRNAs has not been clarified. Recently genes designated as Hu family have been identified, and their products have been shown to bind to the AU-rich element of 3'-UTR of several genes including VEGF mRNA (Ma et al. 1996; Nabors et al. 2001; Goldberg-Cohen et al. 2002; Iida et al. 2002). It is suggested that the proteins of Hu family changes the VEGF mRNA conformation so that the mRNA is not attacked by RNAase. Alterations in a few nucleotides in the 3'-UTR, such as single nucleotide polymorphism and triplet nucleotide repeat, have been shown to be associated with the deregulation of affected genes (Ramamoorti et al. 2001). Thus, it is suggested that the nucleotide polymorphisms in the 3'-UTR may alter the mRNA conformational integrity, resulting in a genetic variation of the VEGF gene expression.

It has been generally accepted that after a solid tumor has attained a few millimeters in diameter, further expansion of the tumor-cell population requires the induction of angiogenesis (Folkman 1990). Our own data (Sato et al. 1994, 1999; Tsuchiya et al. 2001) and others (Brown et al. 1993; Takahashi et al. 1994; Nicol et al. 1997) have demonstrated that RCC is a highly angiogenic-dependent tumor, and that VEGF is a crucial mediator of the angiogenesis. Various genetic and epigenetic events are involved in the up-regulation of VEGF gene (Ferrara 2001). Renner et al. (2000) demonstrated that for the polymorphism at *C936T* in the 3'-UTR of VEGF gene, *CC* genotype is associated with higher plasma VEGF level as compared with *CT* and *TT* genotypes while no specific genotype of the polymorphism at *G1612A* influences the plasma VEGF level. If this is the case, the *C936T CC* genotype in the 3'-UTR of VEGF gene might facilitate angiogenesis in the small foci of RCC and lead to clinical manifestation. It is also speculated that tumor cells having this polymorphism would have an advantage of further expansion, and the *C936T* genotype

would be a marker to identify individuals susceptible to RCC.

To our knowledge, our study is the first to investigate the relationship between the polymorphisms, *C702T*, *C936T* and *G1612A*, in the 3'-UTR of VEGF gene and the risk of RCC by PCR-RFLP analysis on native Japanese subjects consisting of 145 RCC cases and 145 healthy controls. However, we failed to demonstrate any significant association between the VEGF polymorphisms and the risk of RCC, despite that all possible combinations of genotypes and stratification of clinicopathological parameters were included into our analyses. These results suggest that the *C702T*, *C936T* and *G1612A* polymorphisms of the 3'-UTR of VEGF gene are not relevant markers to identify the individuals susceptible to RCC.

Recently, other nucleotide polymorphisms have been found in the promoter region and 5'-UTR of VEGF gene. Of them, *G(-1154)A* polymorphism in the promoter region has been associated with higher VEGF production (Shahbazi et al. 2002) in recipients with acute renal rejection or with the risk of prostate cancer (McCarron et al. 2002). The *AA* genotype is suggested to be a low producer of VEGF and thus is a protective factor for prostate cancer. While, Awata et al. (2002) has reported that *G(-634)A* polymorphism in the 5'-UTR is associated with an elevated serum VEGF level. These polymorphisms should be further investigated with respect to their association with the susceptibility and progression of renal cell cancer.

In conclusion, the present study indicated that there are considerable ethnic differences in the *C702T* and *G1612A* genotype prevalence and suggested that the polymorphisms at *C936T* and *G1612A* in the 3'-UTR of VEGF gene are not associated with the risk of RCC. The other nucleotide polymorphisms of this gene could be a candidate responsible for the susceptibility and/or progression of this tumor.

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