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Original Article

The *TERT* locus genotypes of rs2736100-CC/CA and rs2736098-AA predict shorter survival in renal cell carcinoma

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Abstract

Objectives: The single nucleotide polymorphisms (SNPs) at the *TERT* rs2736100 and rs2736098 are associated with multicancer susceptibility, however, published findings regarding renal cell carcinoma (RCC) risk are conflicting. In addition, the potential of these SNPs to predict outcomes in RCC remains unclear. The present study is designed to address these questions.

Patients and Methods: We recruited 343 patients with RCC and ethnic-/sex-matched healthy controls. *TERT* rs2736100 and rs2736098 SNPs were analyzed, and their relationships with relapse/survival were evaluated using univariate or multivariate Cox regression.

Results: The genotype distribution did not significantly differ between RCC patients and healthy controls. RCC patients carrying the rs2736100-CC/CA variants had significantly shorter progression-free and overall survival (PFS and OS) than did those AA-carriers ($P = 0.009$ and 0.032, respectively), while the rs2736098-AA variant was associated with shorter PFS and OS ($P = 0.008$ and 0.017, respectively). Multivariate analyses showed that rs2736100-CC/CA and rs2736098-AA predicted shorter PFS and OS independently of other established prognostic variables in RCCs. Furthermore, patients carrying both rs2736100-CC/CA and rs2736098-AA had shortest PFS and OS ($P = 0.003$ and 0.013, respectively) and the hazard ratio of relapse was 7.2 (95% confidence interval: 2.0−26.1).

Conclusions: There is no significant association between rs2736100/rs2736098 SNPs and RCC risk. rs2736100-CC/CA and rs2736098-AA variants serve as independent predictors of a poor prognosis in RCC. Given that blood or even urinary DNA can be used to genotype these germline variants before treatment, these 2 SNPs may serve as a potential marker for risk stratification.

Keywords: Renal cell carcinoma; Prognostic factors; SNPs; *TERT*; Telomerase

Abbreviations: CI, confidence interval; PFS, progression-free survival; HC, healthy control; HR, hazard ratio; OR, odds ratio; OS, overall survival; RCC, renal cell carcinoma; SNP, single nucleotide polymorphism; *TERT*, telomerase reverse transcriptase

1. Introduction

Renal cell carcinoma (RCC) is the most common form of cancer that originates in the kidneys and leads up to 150,000 yearly deaths worldwide [1−3]. As a pathologically and genetically heterogeneous disease, RCC is categorized into at least 4 different subtypes among which clear cell RCC (ccRCC) is the commonest one constituting approximately

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80% [1,2]. ccRCC arises from the proximal tubular epithelium, and inactivation of the von Hippel−Lindau disease tumor suppressor gene (*VHL* gene) occurs widely, which is believed to be an early or a first event during the oncogenic process of ccRCC [4]. However, genetic events or molecular mechanisms underlying the full development of ccRCC remain poorly understood [4]. Clinically, disseminated tumors manifest in one-third of RCC patients at diagnosis, and a fraction (20%) of patients may undergo metastatic progression as early as 1 month following surgery [3−5]. Moreover, recurrent RCC responds poorly to the current systemic therapy. Although a number of molecular and clinical variables have been tested as prognostic markers in RCC, there is still a lack of apt predictors of prognosis in this disease. By improving our understanding of RCC pathogenesis, the identification of reliable prognostic factors would be facilitated.

Telomerase is a RNA-dependent DNA polymerase with telomerase reverse transcriptase (*TERT*) as the catalytic key component [6−8]. Telomerase is responsible for lengthening telomeric DNA and essential to maintain genomic stability and oncogenesis. The *TERT* gene is transcriptionally repressed and telomerase is silent in the majority of normal human somatic cells, while *TERT* induction coupled with telomerase activation is required for malignant transformation by stabilizing telomere length [6−8]. Given the fundamental role of telomerase/*TERT* in oncogenesis, much attention has been paid to mechanisms behind telomerase activation during oncogenesis. More recently, genome-wide association studies and other genetic analyses revealed that a panel of single nucleotide polymorphisms (SNPs) of the *TERT* gene are intimately associated with intrinsic telomere length and cancer susceptibility, among which rs2736100 (located in *TERT* intron 2) and rs2736098 (*TERT* exon 2) are most studied [9−18]. The variants at these 2 loci are associated with risk of multiple-types of cancer, as documented in many published reports [11]. However, little is known about their relation to RCC susceptibility, and moreover, it is currently unclear whether they can predict outcome and potentially guide treatment decisions in patients with RCC or other malignancies. The present study is thus designed to address these questions with a focus on RCCs.

Table 1
Characteristics of 343 patients with RCC

	Total (Number = 343)
Gender (%)	
Male	233 (67.9)
Female	110 (32.1)
Age, years (median, IQR)	57 (49−66)
Tumor stage (%)[b]	
1	288 (84.0)
2	18 (5.2)
3	30 (8.7)
4	3 (0.9)
Missing data	4 (1.2)
Lymph nodes stage (%)[b]	
0/X	287 (83.7)
1	38 (11.1)
Missing data	18 (5.2)
Metastasis stage (%)[b]	
0	329 (95.9)
1	12 (3.5)
Missing data	2 (0.6)
Pathology (%)	
Clear cell	313 (91.3)
Papillary	15 (4.4)
Chromophobe	14 (4.1)
Unclassified	1 (0.3)
Fuhrman nuclear grade (%)	
1	31 (9.0)
2	210 (61.2)
3	80 (23.3)
4	5 (1.5)
Unknown[a]	17 (5.0)
Tumor size, cm (median, IQR)	3.5 (2.6−5.0)
Microvascular invasion (%)	20 (6.4)
Surgical treatment (%)	
Nephron sparing surgery	145 (42.3)
Radical nephrectomy	190 (55.4)
Radical nephrectomy + thrombectomy	8 (2.3)
Systemic targeted therapy (%)	16 (4.7)
Radiotherapy (%)	2 (0.6)
Chemotherapy (%)	7 (2.0)
Surgery year (%)	
2007−2011	153 (44.6)
2012−2016	190 (55.4)

IQR = interquartile range; RCC = renal cell carcinoma.
[a] Due to the chromophobe RCC (14), the unclassified RCC (1), and the missing data (2).
[b] According to the 2010 UICC/AJCC TNM staging system for RCC (7th edition).

2. Patients and method

2.1. Study populations

Three hundred and forty-three newly diagnosed, histologically confirmed sporadic RCC patients were recruited from the Department of Urology, Peking University Third Hospital during a ten-year (2007−2016; 2007−2011 $n = 153$; 2012−2016 $n = 190$) period, as shown in Table 1. Patients with localized RCC underwent nephron sparing surgery or radical nephrectomy. Patients with metastatic disease underwent nephrectomy and adjuvant therapy (Table 1). After surgery, these patients were regularly followed up. The adult healthy controls (HCs) were recruited from the Medical Examination Center, Shandong University Second Hospital and was ethnic-/sex-matched for rs2736100 (343 HCs) and rs2736098 (313 HCs) genotyping. The ethnic background of both RCC patients and HCs was Han Chinese. Patients' tumor specimens and HCs' blood samples were obtained from the participants with informed consent. The study was approved by the Peking University Third Hospital and the Second Hospital of Shandong University Ethics Committees. The study was performed in accordance with relevant ethical guidelines and regulations.

2.2. DNA extraction and genotyping

Genomic DNA was extracted using QIAGEN DNA extraction kits, as described [15,16]. The rs2736100 and rs2736098 genotyping was performed using predesigned TaqMan SNP genotyping assay kits on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems). Positive and negative controls were both included in each assay with the following condition: 95˚C for 10 minutes, followed by 40 cycles of 92˚C for 15 seconds and 60˚C for 1 minute.

2.3. RCC cases from the Cancer Genome Atlas (TCGA)

TERT mRNA expression and survival information for RCC cases (538 ccRCC; 290 papillary RCC, and 66 chromophobe RCC) in the TCGA database were downloaded at cBioPortal for cancer genomics [19,20], on September 14, 2018, and used for analysis of *TERT* expression and its association with progression-free and overall survival (PFS and OS) in RCC patients. The median separation of *TERT* mRNA levels was utilized to define low and high *TERT* groups of RCC patients.

2.4. Statistical analyses

The evaluation of distribution differences of selected variables and genotype/allele of the *TERT* polymorphisms between patients and HCs were done using χ^2 test. Hardy–Weinberg equilibrium of the genotype distribution among the controls was tested by a goodness-of-fit χ^2 test. Unconditional univariate logistic regression analyses were used to estimate odds ratios with 95% confidence interval (CI) for RCC susceptibility. PFS and OS were visualized with Kaplan–Meier plots, and *P* values were calculated by log-rank test. Univariate Cox regression analysis was first performed and then significant factors were included in multivariate analysis to define independent prognostic predictors and hazard ratios with 95% CI. All the tests were computed using SigmaStat3.1 software (Systat Software, Inc., Richmond, CA). The *P* values < 0.05 were considered as statistically significant.

3. Results

3.1. RCC patients

A total of 343 patients with RCC were included and clinico-pathological characteristics of these patients, including age, gender, tumor size, clinical stage, lymph node and metastasis status, and pathologic/histological features, are summarized in Table 1. Clinical stages were evaluated according to the 2010 union for international cancer control (UICC)/American Joint Committee on Cancer (AJCC) tumour node metastasis (TNM) staging system for RCC (7th edition). The information on adjuvant therapy is documented in Table 1. A total of 16 (4.7%) patients received target therapy, among whom, 11 received sorafenib and 5 received sunitinib. At a median follow-up of 51 months, a total of 34 (9.9%) patients recurred, and 24 (7.0%) patients died.

3.2. rs2736100 and rs2736098 SNPs are not significantly associated with RCC susceptibility

The genotyping results of *TERT* rs2736100 A > C and rs2736098 C > A in both HCs and RCC patients are documented in Supplementary Tables S1 and S2, respectively. The genotype/allele distribution of rs2736100 and rs2736098 SNPs in HCs was consistent with the published genotyping data obtained from Han Chinese [15,16]. There were no significant differences in either genotypes or alleles of either rs2736100 or rs2736098 SNPs between HCs and RCCs, which suggests the lack of an association between these variants and RCC risk.

3.3. The rs2736100-CC and CA variants predict shorter PFS and OS for RCC patients

We then sought to evaluate whether the SNPs at rs2736100 could serve as a prognostic factor. First, all the 343 patients were categorized into 3 groups based on their rs2736100 genotypes, and their survival time was compared. Univariate log-rank test of the rs2736100-CC variant predicted a significantly shorter PFS and OS ($P = 0.032$ and 0.025, respectively, Fig. 1A). Because similar survival rates were observed in patients with CC and CA variants, we further divided these patients into 2 groups: AA and CC/CA carriers, and their survival was then evaluated. As shown in Fig. 1B, the rs2736100-AA genotype was significantly associated with longer PFS and OS compared to CC/CA variants ($P = 0.009$ and 0.032, respectively). To further evaluate the independent prognostic significance of the rs2736100-CC/CA genotypes, we performed univariate (as shown in Table 2) and multivariate analysis of PFS and OS by including the established RCC prognostic variables (age, stage, grade, microvascular invasion, and metastasis). The multivariate results documented in Table 3 clearly showed the rs2736100 CC/CA as an independent prognostic factor in RCC.

3.4. The rs2736098-AA genotype predicts shorter PFS and OS for RCC patients

We further evaluated whether the rs2736098 SNP was also associated with RCC survival. Initial analyses were similarly performed by dividing patients into 3 groups based on their genotypes. AA-carriers exhibited shorter OS with a border line *P* value ($P = 0.053$), whereas significantly shorter PFS ($P = 0.023$) (Fig. 1C). Similar survival proportions were observed in patients with CC and AC variants, and therefore we combined these 2 groups together and

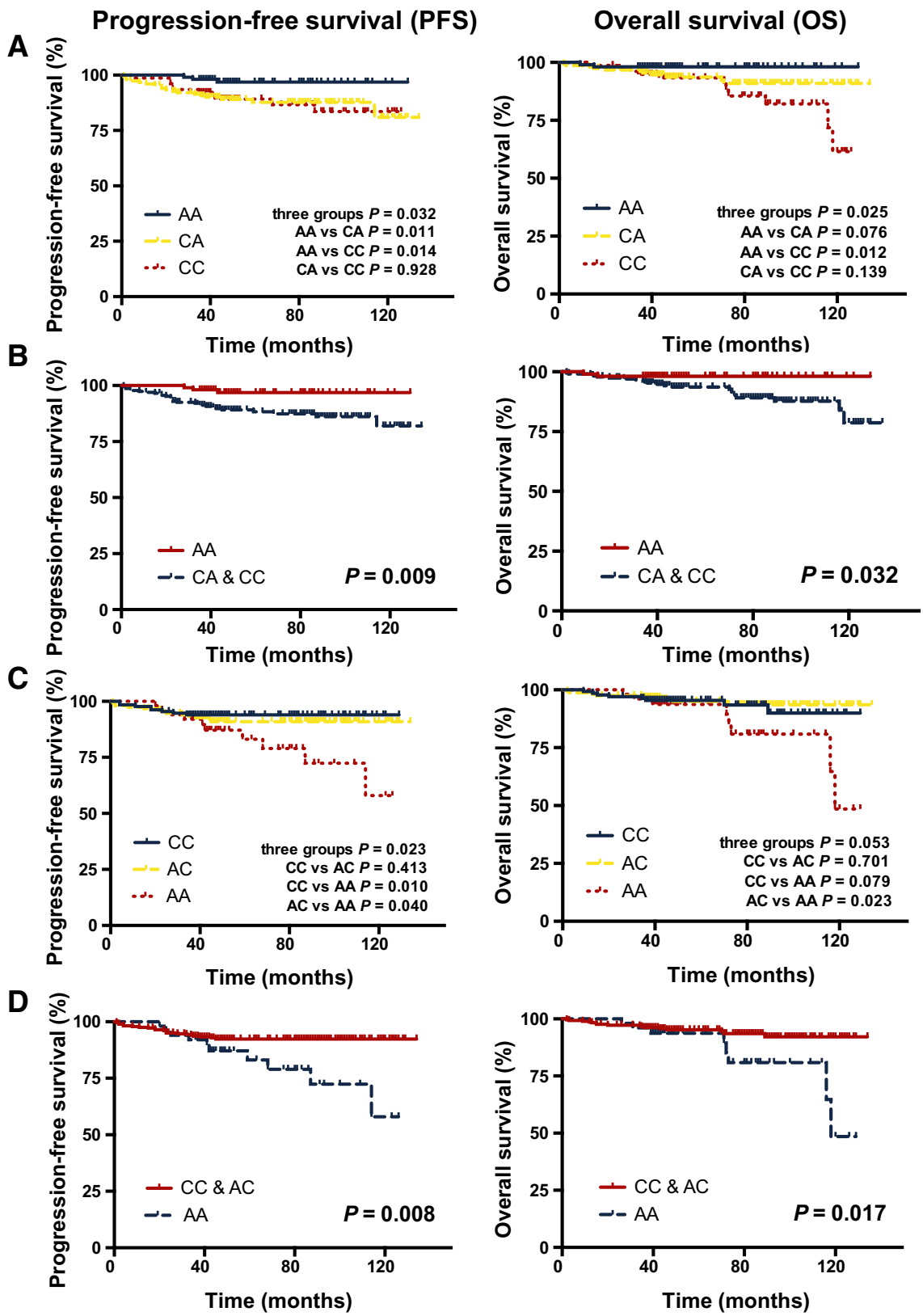


Fig. 1. ***TERT* rs2736100 and rs2736098 SNPs are associated with overall and disease-free survival (OS and PFS) in patients with renal cell carcinoma (RCC).** (A and B): The rs2736100-CA/CC variants predict shorter OS and PFS in RCCs. (C and D): The rs2736098-AA variant predicts shorter OS and PFS in RCCs.

Table 2
Univariate analyses of *TERT* SNPs and clinico-pathological variables for RCC patient survival

	Progression-free survival		Overall survival	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	1.01 (0.98−1.04)	0.438	1.07 (1.03−1.11)	0.001
Gender		0.182		0.283
Male	1.0 (ref.)		1.0 (ref.)	
Female	0.57 (0.25−1.31)		0.58 (0.22−1.56)	
Tumor stage[a]		0.002		0.046
1 & 2	1.0 (ref.)		1.0 (ref.)	
3 & 4	3.45 (1.55−7.64)		2.73 (1.02−7.32)	
Lymph nodes stage[a]		0.160		0.801
X/0	1.0 (ref.)		1.0 (ref.)	
1	1.90 (0.78−4.65)		1.17 (0.34−4.00)	
Metastasis stage[a]		<0.001		0.001
0	1.0 (ref.)		1.0 (ref.)	
1	7.68 (2.95−19.97)		6.73 (2.28−19.90)	
Pathology		0.591		0.496
ccRCC	1.0 (ref.)		1.0 (ref.)	
Non-ccRCC	0.68 (0.16−2.82)		0.50 (0.67−3.70)	
Microvascular invasion		0.063		0.009
No	1.0 (ref.)		1.0 (ref.)	
Yes	2.72 (0.95−7.81)		4.34 (1.45−12.99)	
Fuhrman nuclear grade		0.078		<0.001
1 & 2	1.0 (ref.)		1.0 (ref.)	
3 & 4	1.88 (0.93−3.78)		4.50 (1.93−10.49)	
Tumor size (cm)	1.37 (1.21−1.55)	<0.001	1.23 (1.04−1.46)	0.017
Surgery year		0.200		0.083
2007−2011	1.0 (ref.)		1.0 (ref.)	
2012−2016	1.64 (0.77−3.52)		2.74 (0.88−8.56)	
rs2736098		0.019		0.002
CC & AC	1.0 (ref.)		1.0 (ref.)	
AA	2.44 (1.16−5.12)		2.70 (1.16−6.33)	
rs2736100		0.012		0.049
AA	1.0 (ref.)		1.0 (ref.)	
CA & CC	4.62 (1.41−15.16)		4.30 (1.01−18.33)	
SNP risk factor		0.010		0.026
0	1.0 (ref.)	ref.	1.0 (ref.)	ref.
1	3.73 (1.11−12.57)	0.034	3.30 (0.75−14.58)	0.115
2	7.17 (1.97−26.09)	0.003	7.36 (1.56−34.82)	0.012

ccRCC = clear cell renal cell carcinoma; CI = confidence interval; HR = hazard ratio; non-ccRCC = nonclear cell renal cell carcinoma; Ref. = reference.
[a] According to the 2010 UICC/AJCC TNM staging system for RCC (7th edition).

then made a comparison between them and the AA-harboring patients. As shown in Fig. 1D, both PFS and OS were significantly shorter in patients with rs2736098-AA genotype ($P = 0.008$ and 0.017, respectively). The multivariate analysis revealed that this AA variant was associated with shorter survival independently of other established RCC prognostic factors again including age, metastasis, microvascular invasion, tumor size and grade (Tables 2 and 4).

3.5. The rs2736098-AA and rs2736100-CC/CA are independent with each other and their combination is more powerful for relapse/survival prediction

We first performed the linkage disequilibrium analysis of rs2736098 and rs2736100 in the Han Chinese population (www.ensembl.com). D' is 0.67 and r2 is 0.41, which indicates a nonsignificant association between these 2 SNPs.

Then, to determine whether rs2736098-AA and rs2736100-CC/CA are independent of each other in predicting survival, we divided the patients into rs2736098-AA and AC/CC groups, and then evaluated the effect of rs2736100 SNPs on the survival of these 2 group patients separately. As shown in Fig. 2A, the rs2736100-CC/CA variants were associated with significantly shorter PFS in patients with rs2736098-AC/CC but not in AA-carriers. We further tested the combined role of rs2736098-AA and rs2736100-CC/CA in predicting patient outcome. Patients carrying both rs2736098-AA and rs2736100-CC/CA (2 risk factors) exhibited the worst prognosis compared to those with only 1 risk factor or no risk factors (Fig. 2B and Table 2, $P = 0.003$ and 0.013 for patient PFS and OS, respectively). Consistently, patients carrying these both factors had a significantly elevated risk to relapse (hazard ratio [95% CI]: 7.2 [2.0−26.1]).

Table 3
Multivariate analyses of rs2736100 SNPs and clinico-pathological variables for RCC patient survival

	Progression-free survival		Overall survival	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	Not included		1.09 (1.04−1.14)	<0.001
Tumor stage[a]		0.068		0.991
1 & 2	1.0 (ref.)		1.0 (ref.)	
3 & 4	1.52 (0.97−2.40)		1.01 (0.33−3.07)	
Metastasis stage[a]		0.007		0.006
0	1.0 (ref.)		1.0 (ref.)	
1	4.71 (1.54−14.37)		7.23 (1.77−29.52)	
Microvascular invasion	Not included			0.089
No		1.0 (ref.)		
Yes		2.91 (0.85−9.99)		
Fuhrman nuclear grade	Not included			0.001
1 & 2		1.0 (ref.)		
3 & 4		4.52 (1.80−11.37)		
Tumor size (cm)	1.18 (1.02−1.37)	0.024	0.97 (0.77−1.21)	0.769
rs2736100		0.007		0.030
AA	1.0 (ref.)		1.0 (ref.)	
CA & CC	5.40 (1.57−18.56)		9.90 (1.25−78.73)	

CI = confidence interval; HR = hazard ratio; Ref. = reference.
[a] According to the 2010 UICC/AJCC TNM staging system for RCC (7th edition).

3.6. The rs2736100 and rs2736098 SNPs are not associated with clinico-pathological characteristics of RCCs

We next evaluated the potential association between these SNPs and clinico-pathological variables. There were no significant differences in rs2736100 and rs2736098 variants regarding patient age, gender, stage, grade, tumor size, metastasis, and histology (Table S3).

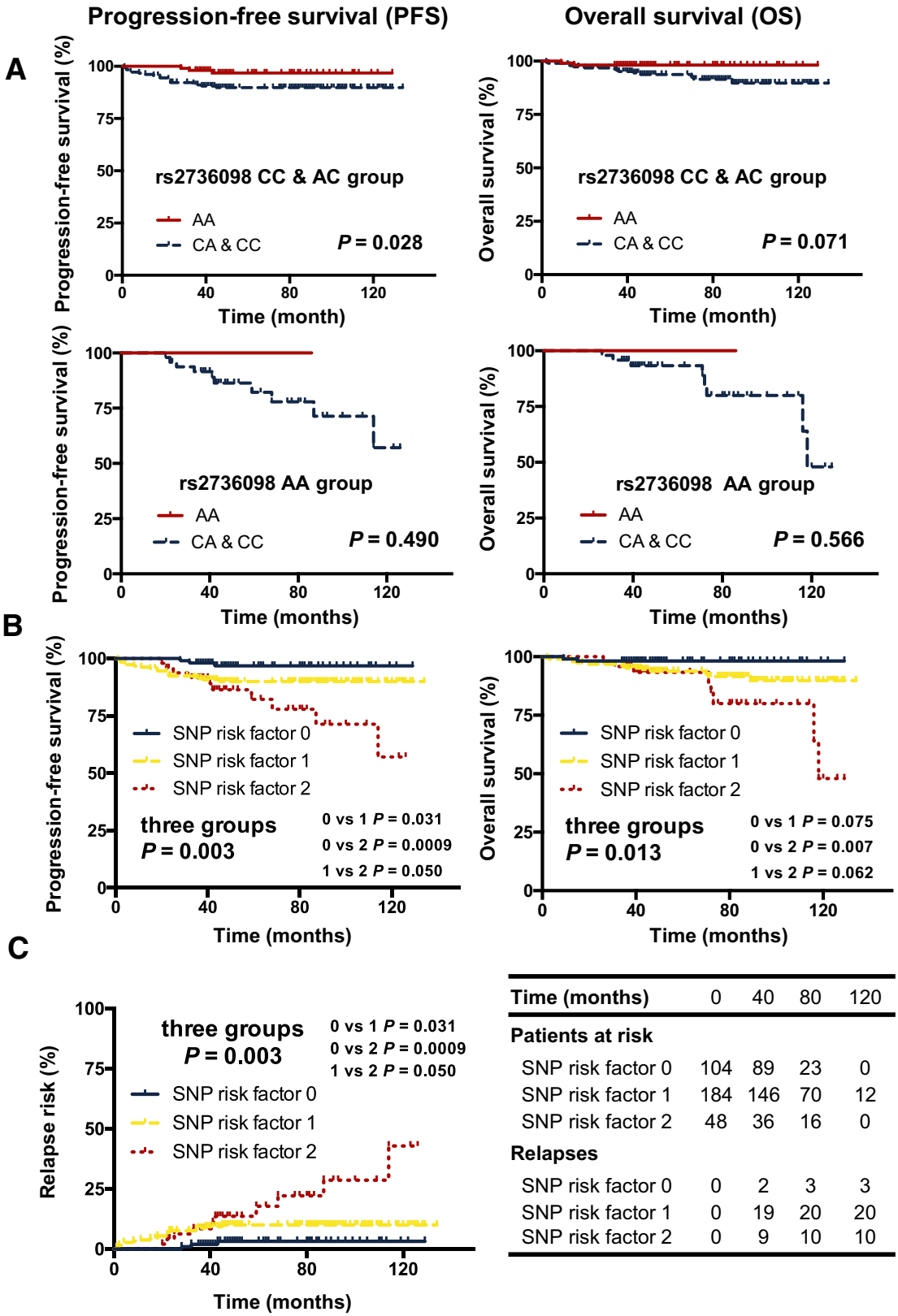
3.7. TERT mRNA expression is associated with PFS and OS in RCCs

Given the above results, together with the previous observations showing that rs2736100-C allele up-regulates *TERT* expression by a stimulatory effect on transcription [12,16], we wanted to examine whether *TERT* expression is associated with RCC patient survival. For this purpose, we

Table 4
Multivariate analyses of rs2736098 SNPs and clinico-pathological variables for RCC patient survival

	Progression-free survival		Overall survival	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	Not included		1.09 (1.04−1.14)	0.001
Tumor stage[a]		0.157		0.979
1 & 2	1.0 (ref.)		1.0 (ref.)	
3 & 4	1.85 (0.79−4.33)		0.99 (0.32−3.10)	
Metastasis stage[a]		0.002		0.006
0	1.0 (ref.)		1.0 (ref.)	
1	5.36 (1.87−15.38)		5.99(1.66−21.62)	
Microvascular invasion				0.096
No	Not included		1.0 (ref.)	
Yes			2.89 (0.83−10.08)	
Fuhrman nuclear grade				0.002
1 & 2	Not included		1.0 (ref.)	
3 & 4			4.19 (1.69−10.41)	
Tumor size (cm)	1.24 (1.09−1.41)	0.001	1.06 (0.86−1.31)	0.605
rs2736098		0.025		0.024
CC & AC	1.0 (ref.)		1.0 (ref.)	
AA	2.48 (1.12−5.46)		2.89 (1.15−7.29)	

CI = confidence interval; HR = hazard ratio; Ref. = reference.
[a] According to the 2010 UICC/AJCC TNM staging system for RCC (7th edition).



Time (months)	0	40	80	120
Patients at risk				
SNP risk factor 0	104	89	23	0
SNP risk factor 1	184	146	70	12
SNP risk factor 2	48	36	16	0
Relapses				
SNP risk factor 0	0	2	3	3
SNP risk factor 1	0	19	20	20
SNP risk factor 2	0	9	10	10

Fig. 2. ***TERT* rs2736100 and rs2736098 SNPs are independent of each other and their combination predicts overall and disease-free survival (OS and PFS) more precisely in patients with renal cell carcinoma (RCC)**. (A) Patients with RCC were divided into rs2736098-CC/AC and AA groups, and the effect of rs2736100-AA and CC/CA on OS or PFS of these 2 group patients were then evaluated separately. (B) rs2736098-AA and rs2736100- CC/CA was defined as 2 risk factors, patients were divided according to lack (0) and with 1 or 2 risk factors and OS or PFS were then analyzed. (C) RCC patients who carried both rs2736098-AA and rs2736100- CC/CA exhibit significantly increased relapse risk.

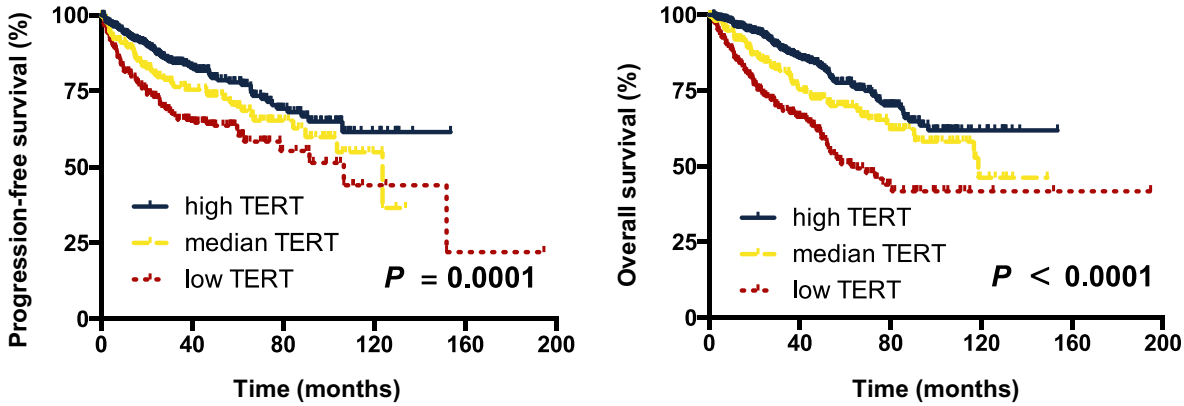


Fig. 3. ***TERT* mRNA expression is associated with overall and disease-free survival (OS and PFS) in the TCGA cohort of patients with renal cell carcinoma (RCC).** RCC patient survival and *TERT* expression data were downloaded via cBioportal. A total of 894 available patients (538 ccRCC; 290 papillary RCC, and 66 chromophobe RCC) with survival and *TERT* mRNA information were divided into 3 groups by upper quartile and median, and OS or PFS were then analyzed.

analyzed the TCGA dataset. A total of 894 patients (538 ccRCC; 290 papillary RCC, and 66 chromophobe RCC) were available and the upper quartile and median were used to separate *TERT* expression levels into 3 groups (Fig. 3). As expected, high levels of *TERT* transcripts predicted both significantly shorter PFS and OS in the TCGA cohort of RCC patients (Fig. 3, $P \leq 0.0001$).

4. Discussion

TERT induction and telomerase activation is essential to malignant transformation. In RCCs, tumor cells utilize various mechanisms to induce *TERT* expression and telomerase activity [4,21,22]. The *TERT* genetic variants have been shown to regulate its own gene expression, and it is thus not surprising to observe an intimate association between *TERT* SNPs and cancer risk. In the present study, we analyzed *TERT* rs2736100 and rs2736098 SNPs in RCC patients. Compared to healthy individuals, the patients did not display significant difference in the distribution of either rs2736100 or rs2736098 genotypes, which may suggest the lack of the involvement of these genetic variants in RCC susceptibility. However, our results do reveal the prognostic value of the rs2736100 and rs2736098 SNPs for patients: rs2736100-CC or CA- and rs2736098-AA-carriers exhibited significantly shorter overall survival time. In particular, these genetic variants are more closely associated with shorter PFS, indicating that these patients developed early RCC relapse.

In order to elucidate how rs2736100/2736098 SNPs affect RCC survival, we analyzed the potential link between them and clinico-pathological variables with established prognostic predictors in RCCs, but failed to observe a correlation with any of those variables, which is consistent with our observation that these 2 SNPs served as independent prognostic factors. It is well established that *TERT* or telomerase promotes cancer development and progression via both telomere lengthening-dependent and independent activities [6,7,31]. Likely, the rs2736100 and rs2736098 SNPs play a role by regulating *TERT* expression. Indeed, Wei et al. observed that the luciferase reporter driven by rs2736100-C allele-containing sequences exhibited a higher activity than that by A allele-carrying fragments in a lung cancer cell line [12]; they also showed significantly higher *TERT* mRNA levels coupled with longer telomere in lung cancer patients harboring the CC genotype. In our recent study on myeloproliferative neoplasms, myeloproliferative neoplasm cells derived from the patients with rs2736100-CC genotype expressed the highest levels of *TERT* mRNA as compared with those from CA- and AA-carriers (CC > CA > AA) [16]. These observations collectively indicate that the rs2736100-C allele contributes to up-regulation of *TERT* expression. We and others have previously demonstrated that *TERT* facilitates cancer cell invasion and metastasis by acting as cofactor to potentiate the transcription of progression-related genes [23–25]. In addition, *TERT* protects cancer cells from apoptosis induced by targeted therapeutic agents or chemotherapeutic drugs [26]. Consistently, the TCGA dataset analysis demonstrated that higher *TERT* expression is associated with significantly shorter PFS and OS in RCCs. Taken together, it is plausible that rs2736100-C-mediated *TERT* up-regulation contributes to relapse risk. On the other hand, however, there has been no clear evidence that rs2736098 SNPs regulate *TERT* expression, and it is even more elusive how rs2736098-AA leads to poor patient outcomes. Further investigations are required to elucidate this puzzle.

In a recent study, de Martino et al. [27] compared the rs2736100 and rs2736098 SNPs between RCC and HCs, and observed that the rs2736098 AA genotype increased the RCC risk, but rs2736100 did not. However, Machiela et al. showed an intimate association between rs2736100-C and RCC susceptibility [17]. In our study, there was no significant association between either rs2736098 or rs2736100 and the RCC risk. The discrepancies need to be elucidated. Of note, European and American Caucasians were studied

in former 2 reports, while our subjects were Han Chinese, and the rs2736098-AA genotype is almost 3-fold more common in European healthy population compared with that in Chinese [28,29]. Intriguingly, the RCC incidence is more than 2-fold higher in Europe than in China [2,30]. Racial or ethnical disparities in disease incidence and pathogenesis have been well documented, and different genetic backgrounds are believed to play important roles, which may provide a potential explanation. However, patients and HCs had the same ethnical background in the studies by de Martino and Machiela, et al. [17,27], and there might exist different mechanisms, which calls for further investigations.

A potential limitation of our study is that the TCGA data from which *TERT* mRNA expression studies were taken did not have the same ethnic makeup as the patient and HC populations in our study. Thus, these parts of data need to be interpreted with caution and further studies about the relationship of *TERT* expression and the genotypes of rs2736098/rs2736100 should be made.

In conclusion, our present results show that the rs2736100-CC/CA and rs2736098-AA genotypes predict significantly shorter PFS and OS in RCC patients, and their predicative effect is independent of well-established RCC prognostic factors including age, clinical stage, histological grade, metastasis status, tumor size, etc. Moreover, the combination of these 2 SNPs predicts patient survival more precisely and patients carrying both rs2736100-CC/CA and rs2736098-AA are at significantly increased risk for relapse. These findings may have important clinical significance if validated in other large cohorts of patients with RCC. Given that blood or even urinary DNA can be used to genotype these germline variants, noninvasive workup for RCC survival prediction might be attractive in future clinical practice.

Conflicts of interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2019.01.014.

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