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Original Article

CHEK2 SNPs predict better prognosis in HBV-related hepatocellular carcinoma patients

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Abstract: Objective: Checkpoint kinase 2 gene (*CHEK2*) is an important mediator of the DNA damage response pathway. Single nucleotide polymorphisms (SNPs) have been shown to influence the developing risk and clinical characteristics in various types of human malignancies. The values of *CHEK2* SNPs in HBV-related hepatocellular carcinoma patients (HCC) were unknown and discussed here. Methods: The expression and prognostic prediction role of *CHEK2* were searched and analyzed in HBV-related HCC patients by GEO database. SNPs in *CHEK2* were genotyped by SNP selection tools, and further assessed their associations with clinical outcomes of 339 HBV-related HCC patients. Results: Patients with a higher *CHEK2* gene expression predicted a worse relapse free survival (RFS). Moreover, those with a variant alleles CC/TT of SNPs rs1547014 and rs738722 had a significantly better prognosis when compared to the patients with CT genotype (P<0.015 for rs1547014, P=0.001 for rs738722), and CC/TT genotype combined with AFP≤400 ng/ml also predicted the best prognosis in HBV-related HCC patients. In stratified analysis, the protective effect of rs1547014 and rs738722 CC/TT genotype was more evident in patients with adverse strata, comparing the patients with favorable strata. Conclusion: *CHEK2* SNPs rs1547014 and rs738722 probably be potential prognostic bio-markers in HBV-related HCC patients.

Keywords: CHEK2, SNP, hepatocellular carcinoma, HBV

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third leading cause of cancer related death worldwide [1]. There are more than 500,000 newly diagnosed HCC patients every year and 50% of the total numbers of cases and deaths happened in China. And among them, most were HBV-related HCC patients [2]. Though advances in treatment, especially in surgical techniques have improved the survival rates of HCC patients that undergo tumor resection, the long term prognosis after surgical resection remains poor [3, 4]. It is well known that multiple clinical factors have been used as indicators for diagnosis and evaluation of HCC, for instance, drinking status, chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections, large tumor size, vascular invasion, positive portal vein thrombosis, serum alpha fetoprotein (AFP), and BCLC stage [5, 6]. Because the indicators for an accurate and precise prediction of HCC course are not enough today, it is urgent to identify potential biomarkers for improving the efficacy of prognosis prediction and the clinical outcomes of HCC patients.

CHEK2 (Checkpoint kinase2) also termed as *Chk2* or *Cds1* is a key cell cycle control gene located on chromosome 22q12.1 [7, 8], which has been reported as a moderate-penetrance, multi-organ cancer susceptibility gene whose alterations increase the risk of different malignancies including breast, colorectal, prostate cancer and even hepatocellular carcinoma [9-12]. Its protein consists of three distinct functional domains: an N-terminal SQ/TQ cluster (SCD), a forkhead associated (FHA) domain, and a C-terminal kinase [10, 13]. It is a nuclear serine/threonine kinase activated by the Ataxia Telangiectasia Mutated (ATM) protein that plays an important role in DNA damage repair system (DDR system) [14].

Single-nucleotide polymorphisms (SNPs) are attractive biomarkers for translational studies due to its easy to detect from blood samples [15]. Preliminary work had identified several intrinsic polymorphisms of the *CHEK2* gene [15-18]. Matthias Simon showed that *CHEK2* SNP rs2017309 A/T was correlated with an adverse prognosis in a large series of glioblastoma patients [17]. And O'Mara reported that *CHEK2* SNP rs8135424 was associated with the decreased risk of endometrial cancer [16]. For *CHEK2*, the existed studies only detected the biological roles in cell cycle arrest and apoptosis *in vitro*, while the prognostic prediction roles of *CHEK2 or CHEK2* SNPs are still little known. Considering the important biological roles of *CHEK2* in HCC, we suggested that the polymorphisms of *CHEK2* may also affect the prognostic predicting role of HBV-related HCC patients.

As we know, no research has been focused on evaluating the prognostic prediction role of *CHEK2* SNPs rs1547014 and rs738722 in HBV-related HCC patients till now. To evaluate the accuracy of the hypothesis that the *CHEK2* SNPs rs1547014 and rs738722 are associated with the prognosis of HBV-related HCC patients, we performed genotyping analysis in 339 newly diagnosed pathologically proven HBV-related HCC patients. And we found that *CHEK2* has a higher expression in HBV-related HCC tissues than adjacent tissues, and the higher expression predicted worse prognosis in HBV-related HCC patients. In addition, we also found that patients with *CHEK2* SNPs rs1547014 and rs738722 CC/TT genotype predicted better prognosis than the patients with CT genotype.

Methods

Gene expression analyses based on the gene expression omnibus database

To investigate the association between *CHEK2* expression and prognosis of HBV-related HCC patients, we searched Gene Expression Omnibus database (GEO accession: GSE14520), and obtained a cohort of 212 HBV-related HCC patients. Gene expression levels were classified as 'higher' if the expression was higher or equal to seventy-five percent, or 'lower' if expression was lower than the Seventy-five percent.

Study population

A total of 339 newly diagnosed pathologically proven HBV-related HCC patients that had undergone surgical resection were recruited by the Affiliated Tumor Hospital of Guangxi Medical University from April 2002 to September 2012. All the 339 HBV-related HCC patients were diagnosed by histopathological examination and the National Comprehensive Cancer Network (NCCN) clinical practice guidelines for oncology. And they were followed up via telephone or hospital visit until death or the last time follow-up was done in September 2014. The patients have a median follow-up time of 53.0 months (range 2-110 months). No patients had a previous cancer diagnosis of any kind by initial screening examination. The clinicopathological characteristics of patients including age, gender, smoking status, drinking status, pathological grade, biobehaviors of cancer, serum AFP level, hepatic cirrhosis, radical resection and use of transcatheter hepatic arterial chemoembolization (TACE) were obtained from medical records and pathological reports. Tumor status was classified according to the Barcelona Clinic Liver Cancer (BCLC) staging system. Child-Pugh class was defined as previously published. Portal vein tumor thrombus (PVTT) was determined as absence or presence. The case endpoint was overall survival (OS), which was calculated from the date of pathological diagnosis/recruitment to death or the end of available follow-up.

SNP selection, DNA extraction and genotyping

SNP selection tools (http://snpinfo.niehs.nih.gov/snpinfo/snp func.htm) were used to select the candidate SNPs in *CHEK2* from the Web. And finally, two SNPs rs1547014 and rs738722 which were located in CHB and Minor allele frequency (MAF) ≥0.10% in HBV-related HCC patients are identified and assessed in this study. Among them, rs738722 was reported to be associated with the esophageal cancer lymph node metastasis. HCC tissues from HBV-related HCC patients were collected after surgical resection and immediately stored at -80°C until DNA extraction. Genomic DNA of HBV-related HCC tissue samples was extracted using a TIANamp Genomic DNA Kit (Tiangen Biotech (Beijing) Co. Ltd., Beijing, China) according to the manufacture's protocol. DNA purity and concentration were determined using

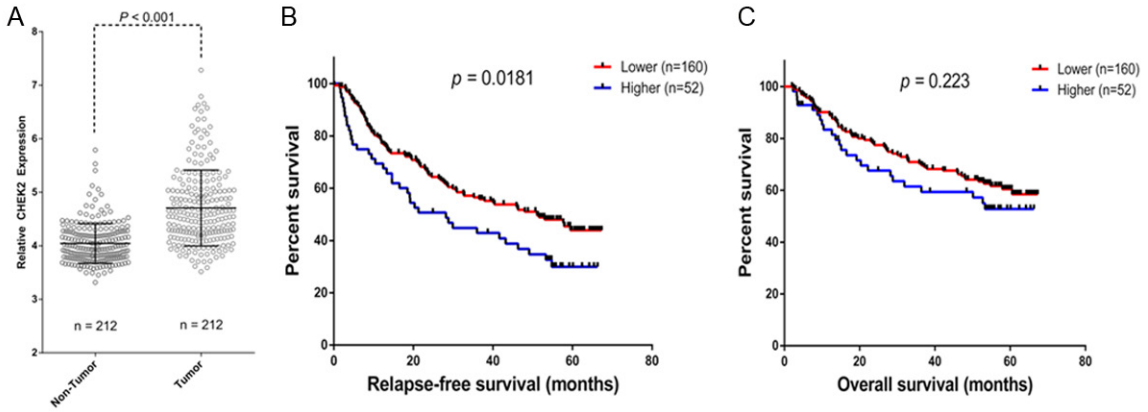

Figure 1. *CHEK2* expression level and its prognostic prediction role in HCC samples from GEO database. A: X^2 analysis showing *CHEK2* has a higher expression in tumor samples than non-tumor samples ($P<0.001$). B: Kaplan-Meier survival curves showing the influence of *CHEK2* expression on Relapse-free survival in HCC patients. C: Kaplan-Meier survival curves showing the influence of *CHEK2* expression on Overall survival in HCC patients.

Table 1. Correlation between CHEK2 gene expression is associated with Relapse-free survival in HBV-related HCC patients

Gene	Patients (n=212)	Overall survival			Relapse-free survival		
		MST (months)	HR* (95% CI)	*P* value*	MRT (months)	HR* (95% CI)	*P* value*
CHEK2							
Lower	160	60.5	Ref.		51.1	Ref.	
Higher	52	53.3	1.19 (0.73-1.94)	0.475	28.2	1.54 (1.18-2.62)	0.033

Note: The 75th percentile of mRNA expression in the total population was used as the cutoff point to define lower and higher expression groups. *Adjustment for age, gender, cirrhosis, BCLC stage, serum AFP levels. Abbreviations: OS, overall survival; RFS, relapse-free survival; MST, median survival time; MRT, median relapse time; HR, hazard ratio; 95% CI, 95% confidence interval; Ref., reference.

NanoDrop 2000 system (Thermo Fisher Scientific, Waktham, MA, USA), and all DNA quality meet with the experimental requirements. PCR was implemented using HotMaster PCR Master Mix (KT208-0 2, Tiangen Biotech (Beijing) Co. Ltd., Beijing, China) and all PCR products were sequenced using the ABIPrism 3730XL (Applied Biosysyems Inc., CA, USA). SNPs rs1547014 and rs738722 were genotyped by Sanger DNA sequencing after PCR amplification using the following primers: rs1547014, forward: TCTCACTTTGTCGCTCAGGC and reverse: GGGAAGGTTCCATT GGATGAT; rs738722, forward: GAA GAATTTGCACTCTGGCC TATG and reverse: AGGACCAAGAACCT GAGG ACCAA.

Statistical analysis

Overall survival (OS) was defined as the time from surgery to death of HCC and replase free survival (RFS) was defined as the time from surgery to disease recurrence. Differences in the distributions of characteristics were evaluated using the Student's t-test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by the Cox proportional hazard model after adjusting for age, gender, AFP level, tumor differentiation and treatment after surgery. Statistical significance was set at a level of 0.05 and all analyses were done using the SPSS version 18.0 (SPSS, Inc., Chicago, IL, U.S.).

Results

CHEK2 has a higher expression in HBV-related HCC tissues and predicted poor prognosis

In recent years, substantial efforts have been made to explore the gene expression profile and potential biomarkers associated with survival of HCC patients. To confirm the expression pattern and prognostic value of *CHEK2* in HBV-related HCC, we queried the GEO database, which allows researchers to correlate gene

Table 2. Correlation between clinicopathological characteristics and overall survival in HBV-related HCC patients

Variables	Patients (n=339)	OS		
		MST (months)	HR* (95% CI)	P*
Age (yr)				
≤49	182	59	Ref.	
>49	157	53	0.97 (0.74-1.25)	0.115
Race				
Han	215	64	Ref.	
Minority	124	49	1.10 (0.84-1.45)	0.571
Gender				
Male	305	55	Ref.	
Female	34	79	0.75 (0.47-1.18)	0.405
BMI				
≤25	275	56	Ref.	
>25	64	58	0.95 (0.68-1.32)	0.638
Drinking status				
No	205	57	Ref.	
Yes	134	51	1.18 (0.91-1.53)	0.325
Smoking status				
No	222	58	Ref.	
Yes	117	53	1.20 (0.91-1.57)	0.253
Adjuvant TACE[a]				
No	160	79	Ref.	
Yes	179	52	1.14 (0.87-1.49)	0.064
BCLC stage				
A	200	90	Ref.	<0.001
B	50	46	1.92 (1.35-2.73)	<0.001
C	89	28	3.10 (2.31-4.16)	<0.001
Cirrhosis				
No	68	84	Ref.	
Yes	271	49	1.21 (0.80-1.84)	0.266
Child-Pugh class				
A	276	64	Ref.	
B	63	31	1.68 (1.21-2.33)	0.030
Antiviral therapy[b]				
No	190	52	Ref.	
Yes	149	83	0.72 (0.53-0.98)	0.006
AFP				
≤300 (ng/ml)	197	67	Ref.	
>300 (ng/ml)	142	45	1.30 (0.99-1.71)	0.049
Radical resection				
No	199	46	Ref.	
Yes	140	68	0.76 (0.59-0.99)	0.031
Pathological grade				
Well	17	77	Ref.	0.561
Moderately	303	48	1.25 (0.68-2.30)	0.491
Poorly	19	41	1.15 (0.40-3.31)	0.497
Tumor size				

expression levels with clinicopathological characteristics. In the GEO cohort, 212 HBV-related HCC tissues paired with 212 adjacent tissues were interrogated to evaluate the mRNA expression level of *CHEK2*. Results showed that *CHEK2* has a higher expression in HBV-related HCC tissues compared to adjacent normal tissues (**Figure 1A**; P<0.001). Then, we analyzed the 212 HBV-related HCC patients, and found that higher expression of *CHEK2* predicted worse RFS (**Figure 1B**; P=0.0181) in HBV-related HCC, while has no effects on OS (**Figure 1C**; P=0.223). Cox regression analysis showed that *CHEK2* mRNA expression was an independent prognostic indicator in HBV-related HCC patients (**Table 1**). These results suggested that *CHEK2* might be a potential prognostic bio-marker for HBV-related HCC patients.

Patient's characteristics and clinical predictors

The clinical and pathologic characteristics of patients are shown in **Table 2**. Overall, 34 female patients and 305 male patients were included. Among them, 215 were Han people and 124 were minority people. The medium survival time was 53 months and 59 month for age ≤49 year and >49 year respectively. As shown in **Table 2**, univariate analysis indicated that patients with BCLC stage B and C (HR=1.92, 95% CI=1.35-2.73; HR=3.1, 95% CI=2.31-4.16, respectively), child-Pugh class B (HR=1.68, 95% CI=1.21-2.33), non-radical resection (HR=0.76, 95% CI=0.59-0.99), non-antiviral therapies (HR=0.72, 95%

≤3 cm	110	99	Ref.	
>3 cm	229	46	2.04 (1.49-2.80)	0.001
No. of tumors				
Single (n=1)	251	64	Ref.	
Multiple (n>1)	88	33	1.61 (1.23-2.12)	0.014
PVTT				
Absence	269	76	Ref.	
Presence	70	28	2.40 (1.12-5.12)	0.007

Note: *HR and *P* value for univariate survival analysis; [a]Adjuvant TACE post hepatectomy; [b]Adjuvant antiviral therapy post hepatectomy. Abbreviations: OS, overall survival; MST, median survival time; HR, hazard ratio; 95% CI, 95% confidence interval; Ref., reference; PVTT, Portal vein tumor thrombus.

CI=0.53-0.98), AFP≥300 ng/ml (HR=1.30, 95% CI=0.99-1.71), tumor size ≥3 cm (HR=2.04, 95% CI=1.49-2.80), multiple tumors (n>1) (HR=1.61, 95% CI=1.23-2.12) and presence of PVTT (HR=2.40, 95% CI=1.12-5.12) had higher risk for death when compared with patients of BCLC stage A, child-Pugh class A, radical resection, antiviral therapies, AFP<300 ng/ml, tumor size <3 cm, single tumor (n=1) and absence of PVTT, respectively. In addition, the clinical features like age, gender, race, BMI, drinking status, smoking status, adjuvant TACE, cirrhosis were found to have no effects on the overall survival of the HBV-related HCC patients in our study.

CHEK2 SNPs rs1547014 and rs738722 predicted better over survival in HBV-related HCC patients

The Cox proportional hazard regression analysis showed that rs1547014 and rs738722 were significantly associated with the of HBV-related HCC patients when adjusted for age, gender, race, smoking status, drinking status, BMI, child-Pugh class, cirrhosis, BCLC stage, pathological grade, TACE status post hepatectomy, antiviral therapy after hepatectomy, radical resection and serum AFP levels (**Table 3**). Results showed that compared to the patients with CT genotype, those with variant alleles CC and TT genotypes had a significantly better OS (**Figure 2A** and **2B**). Adjusted survival curve showed a significant difference in OS between patients with the CC and TT genotypes of SNPrs1547014 and rs738722 and those with CT alleles (*P*<0.001, HR=0.47, 95% CI=0.35-0.64; *P*=0.001, HR=0.58, 95% CI=0.43-0.77) (**Table 4**). The median survival time was 35 months in patients with the CT genotype and 70/72 months in patients with CC/TT genotypes for SNP rs1547014 and rs738722, respectively.

The prognostic predicting role of CHEK2 SNPs combined with serum AFP level on overall survival in HBV-related HCC patients

In this study, we further analyzed the combined effect of *CHEK2* SNPs and serum AFP level on OS of HBV-related HCC patients. According to the SNPs status and serum AFP level, patients were classified as 4 groups: AFP≤400 ng/mL with CC/TT genotype, AFP≤400 ng/mL with CT genotypes, AFP>400 ng/mL with CC/TT genotype and AFP>400 ng/mL with CT genotypes. Multivariate Cox regression analysis indicated that, as compared to patients with CC/TT genotype and low serum AFP (AFP≤400 ng/ml), patients with the CT genotype or a high serum AFP (AFP>400 ng/mL) had a significantly higher risk for death (*P*<0.001) (**Figure 3A**). Similar results were also observed when the rs738722 and serum AFP level were associated simultaneously (**Figure 3B**; **Table 5**).

Stratified analysis on association of CHEK2 SNPs with the survival of HBV-related HCC patients

In our study, a stratified analysis was performed to evaluate the associations of rs1547014 and rs738722 genotypes with HBV-related HCC survival by age, smoking status, serum AFP level, BCLC stage, child-Pugh class, drinking status, smoking status, TACE status, antiviral therapies and radical resection. And we found that patients with rs1547014 CC/TT genotype had a worse OS when they were older than 49 years, AFP>400 ng/ml, BCLC stage C, child-Pugh class B, smoking status, drinking status, take TACE, no antiviral therapy or take radical resection (**Figure 4A**). Except these, the similar results were also observed in the stratified analysis of *CHEK2* SNP rs738722 and the survival of HBV-related HCC patients (**Figure 4B**).

Discussion

In this study, we explored the expression pattern and prognostic prediction role of *CHEK2* gene by the GEO database, and furthermore

Table 3. Effects of SNPs rs1547014 and rs738722 on overall survival in HBV-related HCC patients

SNP	Chr	Position	Gene	Allele	Function	MAF	Overall survival	
							Log-rank P	Cox P
rs1547014	22	29100711	CHEK2	T/C	Intron	0.14	6.39×10^{-5}	0.009
rs738722	22	29130012	CHEK2	T/C	Intron	0.18	0.001	0.011

Note: Cox P adjustment for age, gender, BMI, race, smoking status, drinking status, child-Pugh class, cirrhosis, BCLC stage, pathological grade, TACE status post hepatectomy, antiviral therapy after hepatectomy, radical resection, serum AFP levels. Abbreviations: SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; OS, overall survival.

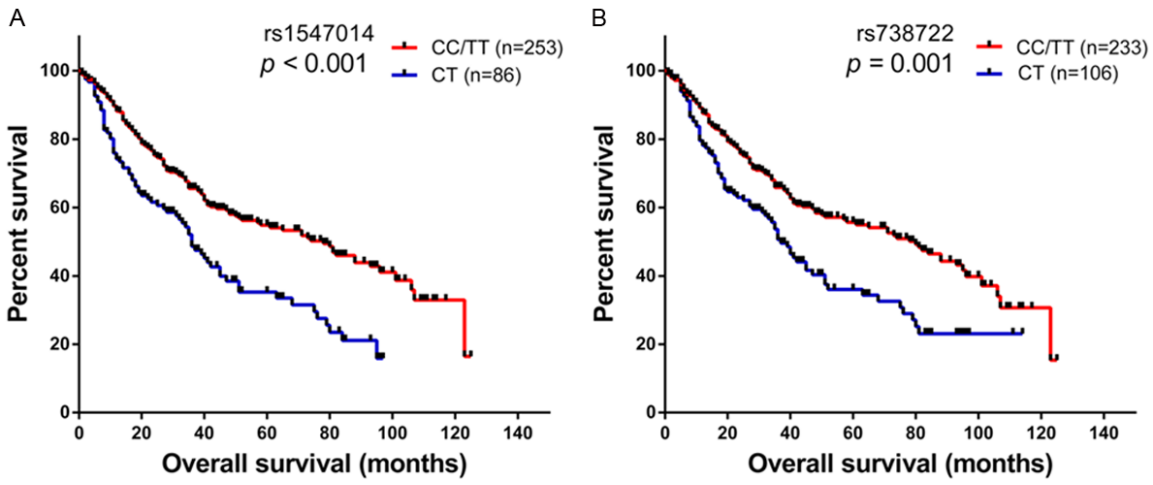

Figure 2. Kaplan-Meier survival curves showing the influence of *CHEK2* SNPs rs1547014 (A) and rs728722 (B) allele status (CC, TT and CT) on overall survival in HBV-related HCC patients.

Table 4. Survival on the basis of genotypes of rs1547014 and rs738722 in HBV-related HCC patients

SNP	Patients (n=339)	Overall survival				
		MST (months)	HR (95% CI)	P	Adjusted HR* (95% CI)	Adjusted P*
rs1547014						
CT	86	35	Ref.	0.001	Ref.	<0.001
TT	5	118	NA	0.928	NA	0.933
CC	248	69	0.059 (0.44-0.77)	<0.001	0.49 (0.40-0.66)	<0.001
CC + TT	253	70	0.57 (0.43-0.75)	<0.001	0.47 (0.35-0.64)	<0.001
rs738722						
CT	106	35	Ref.	0.002	Ref.	0.001
TT	8	92	0.40 (0.15-1.10)	0.077	0.45 (0.16-1.25)	0.125
CC	225	72	0.63 (0.48-0.83)	0.001	0.58 (0.44-0.78)	<0.001
CC + TT	233	72	0.62 (0.48-0.82)	0.001	0.58 (0.43-0.77)	<0.001

Note: *Adjustment for age, gender, BMI, race, smoking status, drinking status, child-Pugh class, cirrhosis, BCLC stage, pathological grade, TACE status post hepatectomy, antiviral therapy after hepatectomy, radical resection, serum AFP levels. Abbreviations: SNP, single nucleotide polymorphism; OS, overall survival; MST, median survival time; HR, hazard ratio; 95% CI, 95% confidence interval; Ref., reference.

examined whether the genetic polymorphisms of *CHEK2* are associated with the overall survival of HBV-related HCC patients. In our study, the most important finding is that the lower *CHEK2* expression predicted a better RFS and *CHEK2* SNPs rs1547014 and rs738722 of CC/TT genotype are significantly associated with a better overall survival compared with CT genotype in HBV-related HCC patients. In addition, a significant association of gene and clinical

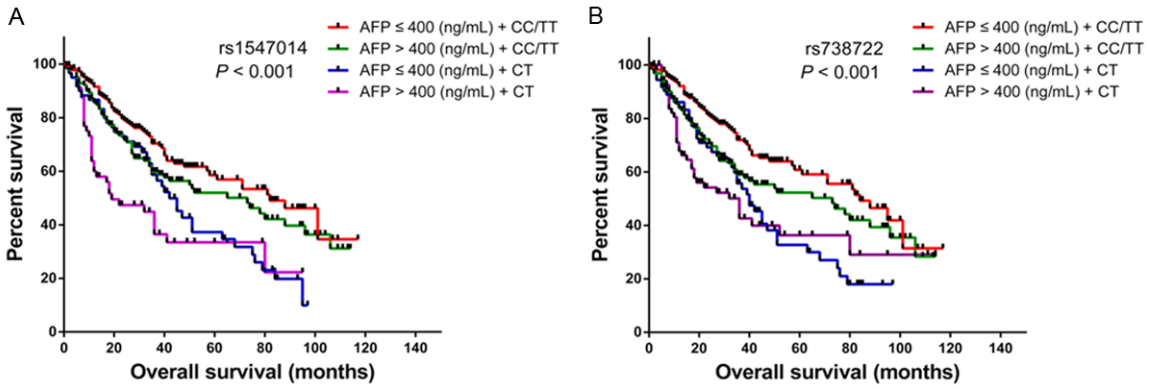

Figure 3. Kaplan-Meier survival curves showing the combined effect of *CHEK2* SNPs rs1547014 (A), rs738722 (B) and serum AFP level on overall survival in HBV-related HCC patients.

Table 5. Combined effects of SNPs (rs1547014, rs738722) and serum AFP level on the overall survival of HBV-related HCC patients

SNP	MST (months)	HR (95% CI)	P	HR* (95% CI)	P*
rs1547014					
AFP≤400 (ng/mL) + CC/TT	81	Ref.	<0.001	Ref.	<0.001
AFP>400 (ng/mL) + CC/TT	64	1.32 (0.94-1.85)	0.109	1.10 (0.74-1.62)	0.641
AFP≤400 (ng/mL) + CT	43	1.83 (1.23-2.70)	0.003	1.84 (1.17-2.91)	0.009
AFP>400 (ng/mL) + CT	21	2.47 (1.63-3.73)	<0.001	2.89 (1.75-4.78)	<0.001
rs738722					
AFP≤400 (ng/mL) + CC/TT	83	Ref.	<0.001	Ref.	0.003
AFP>400 (ng/mL) + CC/TT	64	1.45 (1.03-2.06)	0.035	1.21 (0.81-1.79)	0.357
AFP≤400 (ng/mL) + CT	41	2.00 (1.35-2.93)	<0.001	1.88 (1.21-2.92)	0.005
AFP>400 (ng/mL) + CT	34	2.09 (1.39-3.14)	<0.001	2.20 (1.34-3.63)	0.002

Note: *Adjustment for age, gender, BMI, race, smoking status, drinking status, child-Pugh class, cirrhosis, BCLC stage, pathological grade, TACE status post hepatectomy, antiviral therapy after hepatectomy, radical resection. Abbreviations: SNP = single nucleotide polymorphism; MST = median survival time; HR = hazard ratio; 95% CI = 95% confidence interval; Ref. = reference.

characteristics was observed in joint analysis. In conclusion, we demonstrated that *CHEK2* gene polymorphisms may serve as an independent prognostic marker for HBV-related HCC patients. And once validated, *CHEK2* SNPs rs1547014 and rs738722 combining with traditional clinical-prognostic factors may be used as new target for the treatment of HBV-related HCC patients.

CHEK2, activated by DNA damage, phosphorylates Cdc25A phosphatase, which results in the inhibition of CDK2-cyclin E complexes, and G1-S cell arrest. It inhibits replicative DNA synthesis during the S phase [19]. *CHEK2* and *p53* are thought to participate in the same biological pathway and p53 protein is one of the downstream targets of CHEK2 kinase in various biological roles [20]. In addition, the CHEK2 protein phosphorylates BRCA1 protein in the same DNA repair pathway, and also has been shown to exert several independent functions in regulation mitotic entry, homology directed repair and apoptosis [8, 11, 20, 21].

CHEK2 were usually considered as a tumor suppressor gene [22]. Rare somatic mutations of *CHEK2* have been identified in a number of cancer types, including lung, ovarian cancers and osteosarcomas [23, 24]. Except these, many functional and molecular epidemiological studies have evaluated the association between genetic variants of *CHEK2* and various cancers. *CHEK2* mutations increase the risk of cancers in several different sites including the breast, prostate, thyroid, colon and kidney [24-28]. Large studies have shown that 1100delC is associated with an increased

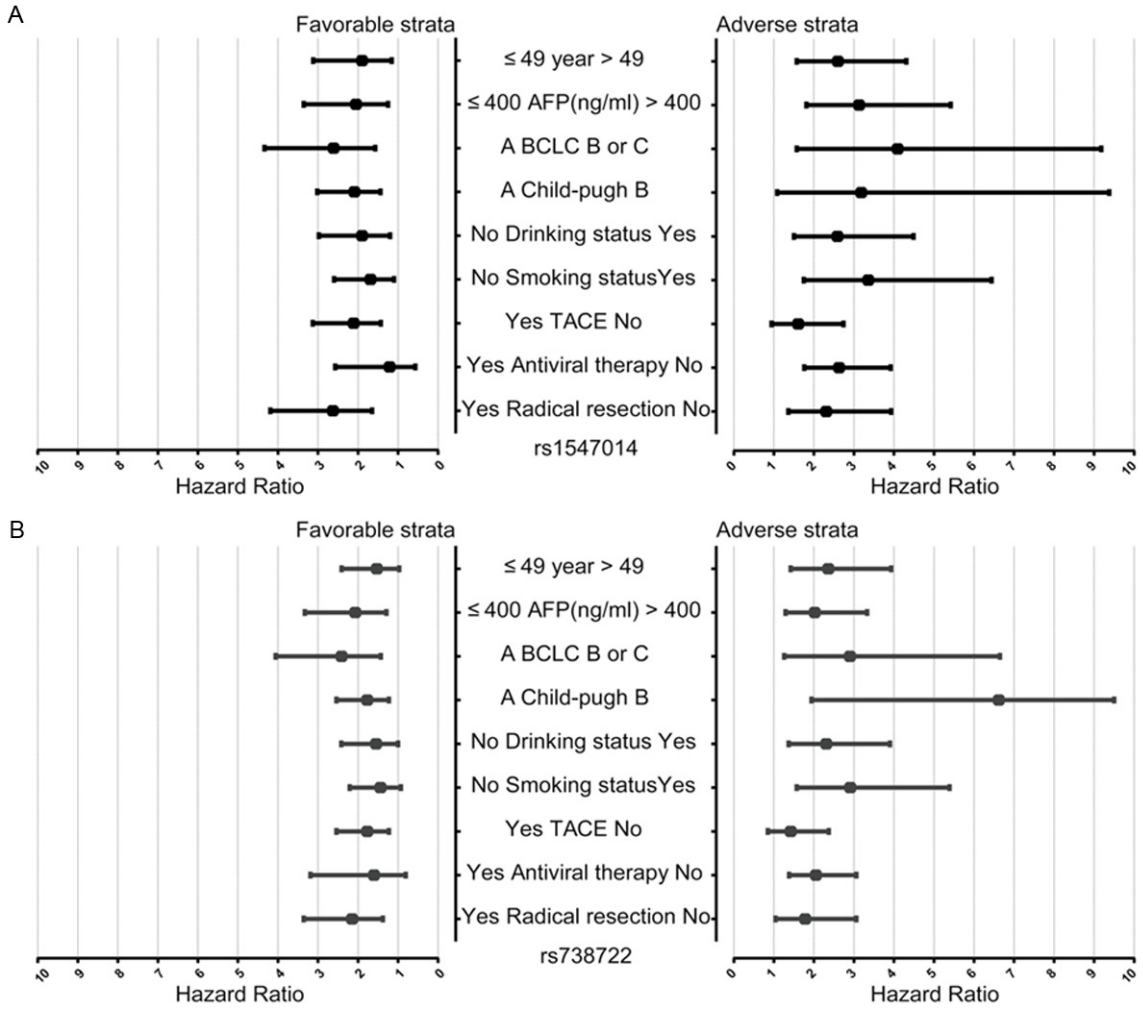

Figure 4. Stratification analysis on the association of rs1547014 (A) and rs738722 (B) with clinical outcome of HBV-related HCC patients. HR was indicated for overall survival, stratified by the favorable and adverse outcomes.

breast cancer risk in many populations [21, 29]. Havranek et al. reported that the germline *CHEK2* mutations affecting protein coding sequence confer a moderately-increased risk of NHL, they are associated with an unfavorable NHL prognosis, and they may represent a valuable predictive biomarker for patients with DLBCL [19]. The polymorphism of *CHEK2* may modulate the binding of transcription factors, and consequently affect the expression and prognostic role of gene [30]. The *CHEK2* rs2236141 variant modifies lung cancer susceptibility in the Chinese population by affecting *CHEK2* expression [30, 31]. Gu et al. reported that the functional variant rs738722 and rs2236142 of *CHEK2* had no effects on the prognosis of patients in esophageal cancer, while contributes to susceptibility of lymph node metastasis in esophageal cancer [31]. Here, our results showed that CT/TT genotypes of SNPs rs1547014 and rs738722 in *CHEK2* were significantly associated with the better prognosis of HBV-related HCC patients, and once validated *CHEK2* SNPs rs1547014 and rs738722 combining with traditional clinical-prognostic factors may be used as new treatment target in HBV-related HCC patients.

From the known reports, we found that the biological behavior and prognostic role of *CHEK2* in various cancers were inconsistent. Our study reported that *CHEK2* higher expression predicted a worse relapse free survival and the CC/TT allele of SNPs rs1547014 and rs738722 were significantly associated with the OS of HBV-related HCC patients independent of main

tumoral prognostic factors, as patients with the CC/TT genotype had a significantly decreased risk of death when compared with those carrying the homozygous CT genotype. And combined the effect of SNPs and serum AFP level on HBV-related HCC patients, those with CC/TT genotype and low serum AFP (AFP≤400 ng/ml) had a significantly lower risk for death. Further stratification analysis indicated that the effect of rs1547014 and rs738722 had more prominence inpatients >49 years old, AFP>400 ng/ml, BCLC stage C, child-Pugh class B, smoking status, drinking status, take TACE, no antiviral therapy or take radical resection. To the best of our knowledge, this is the first evaluation of the potential association between *CHEK2* SNPs rs1547014 and rs738722 and the survival of HBV-related HCC patients. Based on the results of our study, the *CHEK2* SNPs rs1547014 and rs738722 might be used to predict the clinical outcomes of HBV-related HCC patients. The limitations of the present study must be noted. First, considering the inconsistent predictive effects of *CHEK2* SNP in various cancers and the cohort size of the present study was relatively small, larger, well-designed, longitudinal follow-up studies and functional evaluation are warranted to confirm our findings. Second, though several clinical and pathologic characteristics showed significant associations with OS, including AFP levels, tumor size, Child-Pugh class, Antiviral therapy, Radical resection, No. of tumors, PVTT and BCLC stage, it is regretful that we failed to collect the information of some of the factors for small proportion of the subjects in our study. Future studies are essential to investigate the role of genetic polymorphisms in HBV-related HCC patients with more complete and comprehensive clinical pathologic characteristics. Furthermore, after detecting the predictive biomarker of SNPs, it is necessary to determine the biological mechanisms through conducting functional studies.

Conclusions

Our study demonstrated that *CHEK2* SNPs rs1547014 and rs738722 are potential prognostic bio-marker for HBV-related HCC patients. More comprehensive studies are needed to evaluate the association between *CHEK2* SNPs rs1547014 and rs738722 and the prognosis of HBV-related HCC patients, as well as the underlying biological mechanisms caused by the *CHEK2* SNPs rs1547014 and rs738722.

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Disclosure of conflict of interest

None.

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