

## Case Control Study

# Relationships between cell cycle pathway gene polymorphisms and risk of hepatocellular carcinoma

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## Abstract

**AIM:** To investigate the associations between the polymorphisms of cell cycle pathway genes and the risk of hepatocellular carcinoma (HCC).

**METHODS:** We enrolled 1127 cases newly diagnosed with HCC from the Tumor Hospital of Guangxi Medical University and 1200 non-tumor patients from the First Affiliated Hospital of Guangxi Medical University. General demographic characteristics, behavioral information, and hematological indices were collected by unified questionnaires. Genomic DNA was isolated

from peripheral venous blood using Phenol-Chloroform. The genotyping was performed using the Sequenom MassARRAY iPLEX genotyping method. The association between genetic polymorphisms and risk of HCC was shown by *P*-value and the odd ratio (OR) with 95% confidence interval (CI) using the unconditional logistic regression after adjusting for age, sex, nationality, smoking, drinking, family history of HCC, and hepatitis B virus (HBV) infection. Moreover, stratified analysis was conducted on the basis of the status of HBV infection, smoking, and alcohol drinking.

**RESULTS:** The HCC risk was lower in patients with the *MCM4* rs2305952 CC (OR = 0.22, 95%CI: 0.08-0.63, *P* = 0.01) and with the *CHEK1* rs515255 TC, TT, TC/TT (OR = 0.73, 95%CI: 0.56-0.96, *P* = 0.02; OR = 0.67, 95%CI: 0.46-0.97, *P* = 0.04; OR = 0.72, 95%CI: 0.56-0.92, *P* = 0.01, respectively). Conversely, the HCC risk was higher in patients with the *KAT2B* rs17006625 GG (OR = 1.64, 95%CI: 1.01-2.64, *P* = 0.04). In addition, the risk was markedly lower for those who were carriers of *MCM4* rs2305952 CC and were also HBsAg-positive and non-drinking and non-smoking (*P* < 0.05, respectively) and for those who were carriers of *CHEK1* rs515255 TC, TT, TC/TT and were also HBsAg-negative and non-drinking (*P* < 0.05, respectively). Moreover, the risk was higher for those who were carriers of *KAT2B* rs17006625 GG and were also HBsAg-negative (*P* < 0.05).

**CONCLUSION:** Of 12 cell cycle pathway genes, *MCM4*, *CHEK1* and *KAT2B* polymorphisms may be associated with the risk of HCC.

**Key words:** Cell cycle pathway genes; Hepatocellular carcinoma; Single nucleotide polymorphism; Case-control study; Genetic susceptibility

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**Core tip:** We analyzed the effects of polymorphisms of 12 cell cycle pathway genes on the risk of hepatocellular carcinoma (HCC) in a large population of 1019 HCC cases and 1138 controls. The results suggest that *MCM4* rs2305952 CC and *CHEK1* rs515255 TC, TT, TC/TT may be significantly associated with a decreased risk of HCC. *KAT2B* rs17006625 GG may increase the risk of HCC.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is a serious threat to human health worldwide. It is the fourth most common cancer and the second leading cause of cancer death, with nearly 746000 deaths per year<sup>[1]</sup>. The incidence of this fatal disease continues to increase. HCC occurrence and development are related to environmental factors, such as infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), cigarette smoking, and alcohol consumption, as well as genetic susceptibility<sup>[2-4]</sup>. Many studies strongly support that single nucleotide polymorphisms (SNPs) of a variety of genes are associated with HCC<sup>[5-7]</sup>. However, the genetic mechanism underlying the inherited component of HCC is still not fully understood.

The cell cycle comprises the events that result in the formation of two daughter cells through division of the parent cell. Cell cycle progression, including cell division, is influenced by three different types of molecules: cyclin, cyclin-dependent kinases, and cyclin kinase inhibitors<sup>[8]</sup>. The associations between the genetic susceptibility of genes which regulate the cell cycle and the risk of cancer are well known. For instance, a polymorphism of the *p27* generates an increased risk of squamous cell carcinoma of the head and neck<sup>[9]</sup>, while polymorphisms of *p27* and *p21* are associated with a significantly increased risk of HCC<sup>[10]</sup>. Other cell cycle pathway genes implicated in cancer include *cyclinD1*<sup>[11]</sup>, *p53*<sup>[12]</sup>, *CHEK2*<sup>[13]</sup> and *P21*<sup>[14]</sup>.

During the last several decades, an increasing number of studies have shown an association between genetic variants, mainly in the form of SNPs, and the risk of cancer, including breast<sup>[15]</sup>, colorectal<sup>[16]</sup>, cervical, and vulvar cancers<sup>[17]</sup>, and HCC<sup>[18]</sup>. Despite investigations into the association of polymorphisms in cell cycle pathway genes with cancer susceptibility<sup>[19,20]</sup>, in the case of HCC this association remains unclear. Therefore, in this hospital-based study we investigated the associations between the polymorphisms of SNPs in cell cycle pathway genes and the risk of HCC.

## MATERIALS AND METHODS

### Study population

For this case-control study, 2327 subjects were consecutively recruited from June 2007 to December 2013. The 1127 HCC patients were from the Tumor Hospital of Guangxi Medical University and were newly diagnosed with HCC based on biochemical ( $\alpha$ -fetoprotein > 20  $\mu$ g/L) and histopathological examinations. None had undergone radiotherapy or chemotherapy before blood sampling. The 1200 controls from the First Affiliated Hospital of Guangxi Medical University consisted of non-tumor patients admitted within the same period of time. Informed

**Table 1** Summarized information of selected single nucleotide polymorphisms in cell cycle pathway genes

Genes	SNPs	Chromosome (position)	Allele	MAF (hapmap-HCB)
MCM4	rs2305952	8 (47962049)	C/T	C = 0.18
YWHAB	rs2425675	20 (44906293)	A/G	A = 0.20
CDKN2A	rs3088440	9 (21968160)	A/G	A = 0.08
TGFB3	rs3917148	14 (75980178)	A/C	C = 0.10
RBL2	rs3929	16 (53490396)	C/G	C = 0.20
RAD21	rs6987652	8 (116870042)	A/G	A = 0.12
SMAD3	rs11556090	15 (67194045)	A/G	G = 0.09
	rs8025774	15 (67190938)	C/T	C = 0.45
KAT2B	rs17006625	3 (20119604)	A/G	G = 0.14
	rs4858770	3 (20152931)	C/T	T = 0.47
MCM7	rs2070215	7 (100099174)	A/G	G = 0.29
	rs2261360	7 (100095370)	A/C	A = 0.37
CDKN1A	rs3176320	6 (36679011)	A/G	G = 0.17
CDC25C	rs3734166	5 (138329634)	A/G	G = 0.38
CHEK1	rs515255	11 (125627250)	C/T	T = 0.44

MAF (minor allele frequency) was derived from HCB population in HapMap website (<http://hapmap.ncbi.nlm.nih.gov/>). SNPs: Single nucleotide polymorphisms.

consent was obtained from all participants, who also agreed to truthfully complete the questionnaires.

### Information and sample collection

General demographic and behavioral information, hematological indices, and data on the patients' age, sex, nationality, drinking habit, smoking habit, HBV infection, and family history of HCC were obtained in face-to-face interviews by trained investigators. Peripheral venous blood was collected in a vacuum EDTA anticoagulant tube from each participant. Genomic DNA was extracted using a standard phenol-chloroform extraction method and stored at -80 °C.

### SNP selection

From the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>), we found three sets of whole genome expression microarray data which were related to HCC (GSE14520, GSE25097, and GSE12941). A total of 3826 different genes were selected using SPSS 16.0 software (SPSS Inc., Chicago, IL, United States) ( $P < 0.05$ ). Gene ontology classification and pathway enrichment analysis were performed by blast2GO and DAVID (<https://david.ncifcrf.gov/>) and 40 cell cycle pathway genes involved in the cellular process were chose. The genotype information was downloaded from Hapmap website (<http://hapmap.ncbi.nlm.nih.gov/>), and functional SNPs were selected using Haploview 4.2 software (Cambridge, MA 02141, United States) based on a function prediction website (<http://snpinfo.niehs.nih.gov/snpfunc.htm>). Referring to the existing literature on these SNPs with HCC, 15 SNPs in 12 genes (MCM4 rs2305952, YWHAB rs2425675, CDKN2A rs3088440, TGFB3 rs3917148, RBL2 rs3929, RAD21 rs6987652, SMAD3 rs11556090, rs8025774,

KAT2B rs17006625, rs4858770, MCM7 rs2070215, rs2261360, CDKN1A rs3176320, CDC25C rs3734166, and CHEK1 rs515255) were selected in this study. Information of selected SNPs is shown in Table 1.

### SNP genotyping

Before genotyping, each DNA sample was quantified using a UV-Vis spectrophotometer Q5000 (Quawell Technology, Inc., United States) and diluted to a final concentration of 50 ng/μL. SNP genotyping was performed using a MassARRAY system (Sequenom, San Diego, CA, United States) and a matrix-assisted laser desorption ionization-time of flight mass spectrometry method according to the manufacturer's instructions. Primers for PCR and extension were designed using the Assay Designer software package (Sequenom). For quality control, 5% of the samples were randomly chosen and genotyped twice for each locus. Among the 1127 patient samples and 1200 control samples, genotyping was successful for all 15 SNPs in both groups, with a success rate of 92.7%. Thus, all 1019 HCC patients and 1138 controls were included in the final analysis.

### Statistical analysis

Statistical analyses were performed using the SPSS 16.0 software (SPSS Inc., Chicago, IL, United States). Continuous variables were evaluated using the two-sample *t*-test. Categorical variables and genotype frequencies between the HCC patients and controls were compared using the Pearson's  $\chi^2$  and Fisher's exact test. Hardy-Weinberg equilibrium (HWE) was evaluated by a goodness-of-fit  $\chi^2$  test to compare the observed genotype frequencies with the expected ones. The association between SNP genotypes and HCC risk was estimated using unconditional logistic regression analysis and an odds ratio (OR) with 95% confidence interval (CI). All statistical tests were two-sided. A *P*-value  $< 0.05$  was considered to indicate statistical significance.

## RESULTS

### Characteristics of the participants

The 2157 unrelated Chinese subjects enrolled in this study included 881 (86.5%) males and 138 (13.5%) females with HCC. The mean age of these patients was  $48.54 \pm 11.44$  years. The control group consisted of 982 (86.3%) males and 156 (13.7%) females, with a mean age of  $48.01 \pm 11.5$  years. The general demographic characteristics and behavior information on the patients and controls are provided in Table 2. There were no significant differences between the HCC patients and the controls in terms of age, sex, and nationality; however, HCC patients had a significantly higher rate of a positive history of HBV infection, a family history of HCC, smoking, and drinking.

**Table 2** General demographic characteristics and behavioral information among hepatocellular carcinoma patients and controls

Variable	HCC patients <i>n</i> = 1019	Controls <i>n</i> = 1138	<i>t</i> / $\chi^2$	<i>P</i> value
Age	48.54 ± 11.44	48.01 ± 11.50	-1.076	0.28
Gender				
Male	881	982	0.013	0.91
Female	138	156		
Nationality				
Han	673	708	3.591	0.17
Zhuang	332	410		
Others	14	20		
Drinking				
Yes	345	145	136.527	< 0.001
No	674	993		
Smoking				
Yes	355	158	130.222	< 0.001
No	664	980		
Chronic HBV infection				
Yes	794	109	1031.687	< 0.001
No	225	1029		
Family history of HCC				
Yes	80	2	86.597	< 0.001
No	939	1136		

HCC: Hepatocellular carcinoma.

**Allele frequencies and genotype distribution**

In the control group, the genotype frequencies of the 15 SNPs, all but *CDKN1A* rs3176320, were in line with the HWE ( $P > 0.05$ ), which indicated that these study participants were from a homogeneous group. The allele frequencies and genotype distribution of SNPs among the HCC patients and controls from this study are listed in Table 3.

**Association analysis of genetic polymorphisms and HCC**

The association between SNPs and the risk of HCC was examined using unconditional logistic regression analysis. According to the crude ORs and their 95% CIs, *SMAD3* rs11556090 AG or AG/GG and *MCM7* rs2070215 GG carried an increased risk of HCC when compared with the wild genotype *SMAD3* rs11556090 AA and *MCM7* rs2070215 AA, respectively. Individuals with *CDC25C* rs3734166 GG or GA/GG and *KAT2B* rs4858770 TT had a lower risk of HCC than those with the wild genotype *CDC25C* rs3734166 AA and *KAT2B* rs4858770 CC, respectively. However, the association disappeared after adjusting for age, sex, nationality, smoking, drinking, family history of HCC, and HBV infection. Using individuals with the wild genotype AA as the reference, individuals carrying the GG variant of *KAT2B* rs17006625 had a higher risk of HCC (adjusted OR = 1.64, 95%CI: 1.01-2.64,  $P = 0.04$ ) after adjusting for confounding factors. In addition, compared with the wild genotypes *MCM4* rs2305952 TT and *CHEK1* rs515255 CC, individuals carrying the CC variant of *MCM4* rs2305952 or the TC, TT, TC/TT

**Table 3** Allele frequencies and genotype distribution of single nucleotide polymorphisms *n* (%)

SNP	Genotype	HCC patients <i>n</i> = 1019	Control <i>n</i> = 1138	$\chi^2$	<i>P</i> value of HWE
rs2305952	TT	801 (78.61)	883 (77.59)	0.04	0.83
	TC	209 (20.51)	238 (20.91)		
	CC	9 (0.88)	17 (1.49)		
rs2425675	GG	632 (62.02)	724 (63.62)	0.96	0.33
	AG	348 (34.15)	374 (32.86)		
	AA	39 (3.83)	40 (3.51)		
rs3088440	GG	750 (73.60)	813 (71.44)	0.19	0.66
	GA	249 (24.44)	300 (26.36)		
	AA	20 (1.96)	25 (2.20)		
rs3917148	AA	773 (75.86)	882 (77.50)	1.32	0.25
	CA	233 (22.87)	235 (20.65)		
	CC	13 (1.28)	21 (1.85)		
rs3929	GG	619 (60.75)	688 (60.46)	0.03	0.86
	GC	349 (34.25)	395 (34.71)		
	CC	51 (5.00)	55 (4.83)		
rs6987652	GG	743 (72.91)	843 (74.08)	0.38	0.54
	AG	251 (24.63)	270 (23.73)		
	AA	25 (2.45)	25 (2.20)		
rs11556090	AA	622 (61.04)	749 (65.82)	0.15	0.70
	AG	352 (34.54)	346 (30.40)		
	GG	45 (4.42)	43 (3.78)		
rs17006625	AA	526 (51.62)	620 (54.48)	0.48	0.49
	AG	412 (40.43)	446 (39.19)		
	GG	81 (7.95)	72 (6.33)		
rs2070215	AA	465 (45.63)	554 (48.68)	< 0.01	1.00
	AG	424 (41.61)	480 (42.18)		
	GG	130 (12.76)	104 (9.14)		
rs2261360	CC	460 (45.14)	484 (42.53)	2.61	0.11
	CA	433 (42.49)	497 (43.67)		
	AA	126 (12.37)	157 (13.80)		
rs3176320	AA	579 (56.82)	687 (60.37)	5.05	0.02
	GA	383 (37.59)	377 (33.13)		
	GG	57 (5.59)	74 (6.50)		
rs3734166	AA	421 (41.32)	421 (36.99)	0.06	0.8
	GA	481 (47.20)	539 (47.36)		
	GG	117 (11.48)	178 (15.64)		
rs4858770	CC	445 (43.67)	465 (40.86)	0.65	0.42
	CT	461 (45.24)	515 (45.25)		
	TT	113 (11.09)	158 (13.88)		
rs515255	CC	408 (40.04)	411 (36.12)	0.29	0.59
	TC	469 (46.03)	553 (48.59)		
	TT	142 (13.94)	174 (15.29)		
rs8025774	CC	313 (30.72)	335 (29.44)	1.32	0.25
	CT	514 (50.44)	547 (48.07)		
	TT	192 (18.84)	256 (22.50)		

HCC: Hepatocellular carcinoma; SNP: Single nucleotide polymorphism; HWE: Hardy-Weinberg equilibrium

variants of *CHEK1* rs515255 had a significantly lower risk of HCC (adjusted OR = 0.22, 95%CI: 0.08-0.63,  $P = 0.01$ ; adjusted OR = 0.73, 95%CI: 0.56-0.96,  $P = 0.02$ ; adjusted OR = 0.67, 95%CI: 0.46-0.97,  $P = 0.04$ ; adjusted OR = 0.72, 95%CI: 0.56-0.92,  $P = 0.01$ , respectively). The associations are shown in Table 4.

**Association between SNPs and HCC risk stratified by behavioral factors**

HBV infection, alcohol intake status, and smoking status are important behavioral factors that can

**Table 4** Associations between single nucleotide polymorphisms with the risk of hepatocellular carcinoma

SNP	Genotype	OR (95%CI) <sup>1</sup>	P value <sup>1</sup>	OR (95%CI) <sup>2</sup>	P value <sup>2</sup>
rs2305952	TT	Reference		Reference	
	TC	0.97 (0.79-1.19)	0.76	0.97 (0.72-1.32)	0.85
	CC	0.58 (0.26-1.32)	0.19	0.22 (0.08-0.63)	0.01 <sup>a</sup>
	TC/CC	0.94 (0.77-1.16)	0.57	0.89 (0.66-1.19)	0.43
rs2425675	GG	Reference		Reference	
	AG	1.07 (0.89-1.28)	0.49	1.92 (0.71-1.20)	0.54
	AA	1.12 (0.71-1.76)	0.63	0.97 (0.51-1.85)	0.93
	AG/AA	1.07 (0.90-1.28)	0.44	0.93 (0.72-1.20)	0.56
rs3088440	GG	Reference		Reference	
	GA	0.90 (0.74-1.09)	0.29	1.02 (0.76-1.35)	0.92
	AA	0.87 (0.48-1.58)	0.64	1.46 (0.62-3.44)	0.38
	GA/AA	0.90 (0.74-1.09)	0.26	1.04 (0.79-1.37)	0.77
rs3917148	AA	Reference		Reference	
	CA	1.13 (0.92-1.39)	0.24	1.18 (0.88-1.59)	0.28
	CC	0.71 (0.35-1.42)	0.33	1.05 (0.41-2.68)	0.92
	CA/CC	1.10 (0.90-1.34)	0.37	1.17 (0.88-1.56)	0.29
rs3929	GG	Reference		Reference	
	GC	0.98 (0.82-1.18)	0.84	0.97 (0.75-1.26)	0.82
	CC	1.03 (0.69-1.53)	0.88	1.39 (0.80-2.42)	0.25
	GC/CC	0.99 (0.83-1.18)	0.89	1.02 (0.79-1.30)	0.90
rs6987652	GG	Reference		Reference	
	AG	1.06 (0.87-1.29)	0.60	0.92 (0.69-1.23)	0.59
	AA	1.14 (0.65-1.99)	0.66	1.26 (0.55-2.88)	0.59
	AG/AA	1.06 (0.88-1.29)	0.54	0.95 (0.72-1.25)	0.71
rs11556090	AA	Reference		Reference	
	AG	1.23 (1.02-1.47)	0.03	1.11 (0.85-1.44)	0.44
	GG	1.26 (0.82-1.94)	0.29	1.02 (0.54-1.91)	0.96
	AG/GG	1.23 (1.03-1.47)	0.02	1.10 (0.85-1.42)	0.47
rs17006625	AA	Reference		Reference	
	AG	1.09 (0.91-1.30)	0.35	1.07 (0.83-1.38)	0.61
	GG	1.33 (0.95-1.86)	0.10	1.64 (1.01-2.64)	0.04 <sup>a</sup>
	AG/GG	1.12 (0.95-1.33)	0.18	1.14 (0.89-1.46)	0.29
rs2070215	AA	Reference		Reference	
	AG	1.05 (0.88-1.26)	0.58	0.95 (0.73-1.24)	0.71
	GG	1.49 (1.12-1.98)	0.01	1.39 (0.93-2.08)	0.11
	AG/GG	1.13 (0.95-1.34)	0.16	1.03 (0.81-1.32)	0.81
rs2261360	CC	Reference		Reference	
	CA	0.92 (0.77-1.10)	0.35	0.84 (0.64-1.09)	0.19
	AA	0.84 (0.65-1.10)	0.21	0.89 (0.60-1.31)	0.55
	CA/AA	0.90 (0.76-1.07)	0.22	0.85 (0.66-1.09)	0.19
rs3734166	AA	Reference		Reference	
	GA	0.89 (0.74-1.07)	0.22	0.92 (0.71-1.21)	0.56
	GG	0.66 (0.50-0.86)	0.002	0.86 (0.59-1.25)	0.43
	GA/GG	0.83 (0.70-0.99)	0.04	0.91 (0.71-1.17)	0.45
rs4858770	CC	Reference		Reference	
	CT	0.94 (0.78-1.12)	0.47	0.96 (0.74-1.24)	0.74
	TT	0.75 (0.57-0.98)	0.04	0.80 (0.54-1.20)	0.28
	CT/TT	0.89 (0.75-1.06)	0.19	0.92 (0.72-1.18)	0.51
rs515255	CC	Reference		Reference	
	TC	0.85 (0.71-1.03)	0.09	0.73 (0.56-0.96)	0.02 <sup>a</sup>
	TT	0.82 (0.63-1.07)	0.14	0.67 (0.46-0.97)	0.04 <sup>a</sup>
	TC/TT	0.85 (0.71-1.01)	0.06	0.72 (0.56-0.92)	0.01 <sup>a</sup>
rs8025774	CC	Reference		Reference	
	CT	1.01 (0.83-1.22)	0.95	0.95 (0.72-1.27)	0.74
	TT	0.80 (0.63-1.02)	0.08	0.94 (0.66-1.32)	0.71
	CT/TT	0.94 (0.78-1.13)	0.52	0.95 (0.73-1.24)	0.69

<sup>1</sup>OR and 95%CI without adjusting for confounding factors; <sup>2</sup>OR and 95%CI after adjusting for age, sex, nationality, smoking, drinking, family history of hepatocellular carcinoma, and HBV infection. <sup>a</sup>*P* < 0.05 was considered statistically significant. OR: Odds ratio; CI: Confidence interval; SNPs: Single nucleotide polymorphisms.

increase the risk of HCC. To account for the role of these factors, a stratified analysis was conducted. Thus, when the patients were stratified, we found that the variant genotype CC of *MCM4* rs2305952

was associated with a significantly lower risk of HCC among HBsAg-positive individuals, non-drinkers, and non-smokers (adjusted OR = 0.25, 95%CI: 0.08-0.80, *P* = 0.02; adjusted OR = 0.19, 95%CI: 0.06-0.60, *P*

**Table 5** Stratified analysis on the association between single nucleotide polymorphism genotype and hepatocellular carcinoma risk according to hepatitis B virus infection status

SNP	HBsAg-positive				HBsAg-negative			
	Case	Control	OR (95%CI) <sup>1</sup>	P value <sup>1</sup>	Case	Control	OR (95%CI) <sup>1</sup>	P value <sup>1</sup>
rs2305952								
TT	624	80	Reference		177	803	Reference	
TC	161	24	0.86 (0.53-1.42)	0.56	48	214	1.05 (0.72-1.52)	0.80
CC	9	5	0.25 (0.08-0.80)	0.02 <sup>a</sup>	0	12	-	1.00
TC/CC	170	29	0.76 (0.48-1.21)	0.25	48	226	0.99 (0.68-1.43)	0.95
rs17006625								
AA	411	60	Reference		115	560	Reference	
AG	323	42	1.15 (0.75-1.76)	0.54	89	404	1.07 (0.77-1.48)	0.68
GG	60	7	1.36 (0.59-3.17)	0.47	21	65	1.79 (1.02-3.12)	0.04 <sup>a</sup>
AG/GG	383	49	1.18 (0.78-1.77)	0.44	110	469	1.17 (0.86-1.59)	0.32
rs515255								
CC	301	39	Reference		107	372	Reference	
TC	377	52	0.93 (0.59-1.46)	0.75	92	501	0.64 (0.46-0.89)	0.01 <sup>a</sup>
TT	116	18	0.81 (0.44-1.50)	0.51	26	156	0.69 (0.36-0.96)	0.03 <sup>a</sup>
TC/TT	493	70	0.90 (0.59-1.37)	0.62	118	657	0.63 (0.46-0.86)	0.003 <sup>a</sup>

<sup>1</sup>OR and 95%CI after adjusting for age, sex, nationality, smoking, drinking and family history of hepatocellular carcinoma. <sup>a</sup>P < 0.05 was considered statistically significant. OR: Odds ratio; CI: Confidence interval; SNPs: Single nucleotide polymorphisms.

**Table 6** Stratified analysis on the association between single nucleotide polymorphism genotype and hepatocellular carcinoma risk according to drinking status

SNP	Drinking				Non-drinking			
	Case	Control	OR (95%CI) <sup>1</sup>	P value <sup>1</sup>	Case	Control	OR (95%CI) <sup>1</sup>	P value <sup>1</sup>
rs2305952								
TT	273	111	Reference		528	772	Reference	
TC	69	33	0.82 (0.44-1.52)	0.53	140	205	1.02 (0.72-1.44)	0.93
CC	3	1	0.51 (0.03-9.74)	0.66	6	16	0.19 (0.06-0.60)	0.004 <sup>a</sup>
TC/CC	72	34	0.81 (0.44-1.49)	0.49	146	221	0.91 (0.65-1.27)	0.57
rs515255								
CC	145	56	Reference		263	355	Reference	
TC	154	71	0.69 (0.40-1.19)	0.18	315	482	0.73 (0.54-0.99)	0.05 <sup>a</sup>
TT	46	18	1.10 (0.50-2.43)	0.82	96	156	0.56 (0.36-0.86)	0.01 <sup>a</sup>
TC/TT	200	89	0.77 (0.46-1.29)	0.31	411	638	0.69 (0.52-0.92)	0.01 <sup>a</sup>

<sup>1</sup>OR and 95%CI after adjusting for age, sex, nationality, smoking, family history of hepatocellular carcinoma, and hepatitis B virus infection. <sup>a</sup>P < 0.05 was considered statistically significant. OR: Odds ratio; CI: Confidence interval; SNPs: Single nucleotide polymorphisms.

= 0.004; adjusted OR = 0.17, 95%CI: 0.05-0.56, *P* = 0.004, respectively). The variant genotypes TC, TT, and TC/TT of *CHEK1* rs515255 were associated with a significantly lower risk of HCC in HBsAg-negative individuals (adjusted OR = 0.64, 95%CI: 0.46-0.89, *P* = 0.01; adjusted OR = 0.69, 95%CI: 0.36-0.96, *P* = 0.03; adjusted OR = 0.63, 95%CI: 0.46-0.86, *P* = 0.003) and in non-drinkers (adjusted OR = 0.73, 95%CI: 0.54-0.99, *P* = 0.05; adjusted OR = 0.56, 95%CI: 0.36-0.86, *P* = 0.01; adjusted OR = 0.69, 95%CI: 0.52-0.92, *P* = 0.01, respectively). Among smokers, those with the TC variant genotype of *CHEK1* rs515255 had a significantly lower risk of HCC (adjusted OR = 0.54, 95%CI: 0.32-0.93, *P* = 0.03), while among non-smokers the risk was significantly lower in those with the TT variant genotype (adjusted OR = 0.60, 95%CI: 0.39-0.94, *P* = 0.03). In addition, the variant genotype GG of *KAT2B* rs17006625 was shown to carry a significantly higher risk of HCC

among HBsAg-negative individuals (adjusted OR = 1.79, 95%CI: 1.02-3.12, *P* = 0.04). These findings are summarized in Tables 5-7 (only significant SNPs are shown).

## DISCUSSION

We performed this case-control study to investigate the associations between the 15 SNPs in 12 cell cycle pathway genes and the risk of HCC. The *KAT2B* rs17006625 GG was associated with an increased risk of HCC. Furthermore, this harmful effect was more marked in HBsAg-negative carriers. Conversely, the *CHEK1* rs515255 TC, TT, TC/TT and the *MCM4* rs2305952 CC were associated with a decreased risk of HCC. In addition, the risk was markedly lower for those who were carriers of *MCM4* rs2305952 CC and were also HBsAg-positive and non-drinking and non-smoking and for those who were carriers of the TC,

**Table 7** Stratified analysis on the association between single nucleotide polymorphism genotype and hepatocellular carcinoma risk according to smoking status

SNP	Smoking				Non-smoking			
	Case	Control	OR (95%CI) <sup>1</sup>	P value <sup>1</sup>	Case	Control	OR (95%CI) <sup>1</sup>	P value <sup>1</sup>
rs2305952								
TT	274	124	Reference		527	759	Reference	
TC	77	32	1.05 (0.58-1.91)	0.87	132	206	0.94 (0.66-1.34)	0.75
CC	4	2	0.54 (0.06-4.97)	0.59	5	15	0.17 (0.05-0.56)	0.004 <sup>a</sup>
TC/CC	81	34	1.01 (0.57-1.82)	0.96	137	221	0.84 (0.60-1.19)	0.33
rs515255								
CC	145	53	Reference		263	358	Reference	
TC	155	84	0.54 (0.32-0.93)	0.03 <sup>a</sup>	314	469	0.81 (0.59-1.10)	0.17
TT	55	21	0.87 (0.41-1.85)	0.72	87	153	0.60 (0.39-0.94)	0.03 <sup>a</sup>
TC/TT	210	105	0.61 (0.67-1.02)	0.06	401	622	0.75 (0.56-1.01)	0.06

<sup>1</sup>OR and 95%CI after adjusting for age, sex, nationality, drinking, family history of hepatocellular carcinoma, and HBV infection. <sup>a</sup>P < 0.05 was considered statistically significant. OR: Odds ratio; CI: Confidence interval; SNPs: Single nucleotide polymorphisms.

TT, TC/TT genotype of *CHEK1* rs515255 and were also HBsAg-negative and non-drinking. No significant associations were observed between other 12 SNPs and HCC risk.

The cell cycle pathway is one of the most important cellular signaling pathways, as it regulates both cell division and apoptosis. DNA damage readily leads to dysregulation of the cell cycle, which is an essential step in the initiation and development of human malignancies<sup>[21-23]</sup>. In the present study, we reported that three SNPs in cell cycle pathway genes (*MCM4*, *CHEK1*, and *KAT2B*) were significantly associated with the risk of HCC.

*MCM4*, a member of the mini-chromosome maintenance family of proteins, which interact with cell cycle checkpoints and recombinant proteins to stabilize the S phase, is essential for the initiation of eukaryotic genome replication<sup>[24,25]</sup>. Several reports have shown that *MCM4* protein is overexpressed in esophageal carcinomas<sup>[26]</sup>, cervical cancer<sup>[27]</sup>, and cervical squamous cell carcinoma<sup>[28]</sup>. In our study, we found that the polymorphism of *MCM4* rs2305952 was associated with a lower risk of HCC. However, the mechanism of *MCM4* polymorphisms in HCC development remains unclear. Ishimi *et al.*<sup>[29]</sup> found that *MCM4* is one of the crucial targets of DNA replication checkpoint and the phosphorylation of *MCM4*, which is caused by the activation of ATR-CHK1 pathway and CDK2, results in the DNA replication through the inactivation of the *MCM4*/6/7 complex. It is also found that *MCM4* mutations may cause tumors by affecting the formation of the *MCM4*/6/7 complex<sup>[30,31]</sup>.

*CHEK1* is a mediator of cell cycle arrest in response to DNA damage. In addition to controlling cell cycle progression<sup>[32]</sup>, it regulates DNA repair<sup>[33]</sup> and coordinates cell survival and death<sup>[34,35]</sup>. It is reported that *CHEK1* plays an important role in the checkpoint of DNA damage and DNA replication through the ATR-CHK1 pathway<sup>[36-38]</sup>. Lin *et al.*<sup>[39]</sup> performed a meta-analysis to explore the association of *CHEK1* SNPs with

breast cancer in patients registered in the database of the Utah Breast Cancer Study. They found that *CHEK1* polymorphisms are significantly associated with the risk of breast cancer. However, in that study common alleles of *CHEK1* are not implicated in breast cancer risk or in the survival of breast cancer patients after meta-analysis. Our results showed an association between the *CHEK1* rs515255 genetic variant and a decreased risk of HCC, after adjusting for age, sex, nationality, smoking, drinking, family history of HCC, and HBV infection. The conflicting results may reflect the different cancers evaluated and/or differences in the study population. This remains to be clarified in further investigations.

*KAT2B*, also known as *PCAF*, encodes the cofactor PCAF (P300/CBP associated factor) of activated nucleoprotein that is important in cell cycle regulation. *KAT2B* induces cell cycle arrest and/or apoptosis by regulating p53 and affects the acetylation and stability of E2F1 in the presence of DNA damage<sup>[40,41]</sup>. Overexpression of PCAF was reported in samples of both central nervous system tumors and Wilm's tumors<sup>[42]</sup>. In addition, an association between *KAT2B* gene polymorphisms and several human diseases and behaviors has been reported. For example, the *KAT2B* SNP rs9829896 is associated with drug abuse in African Americans<sup>[43]</sup>. We also found that the risk of HCC was higher in individuals with the *KAT2B* rs17006625 GG genotype than with the AA genotype, after adjusting for age, sex, nationality, smoking, drinking, family history of HCC, and HBV infection.

HBV infection status, drinking status, and smoking status are well known to influence the occurrence and development of HCC<sup>[44-47]</sup>. Moreover, some genotypes have no effect on HCC risk when considered within a population as a whole, but the subgroup analysis may show an effect on HCC risk among alcohol drinkers and/or smokers<sup>[48,49]</sup>. Therefore, in our study, we evaluated the role of risk factors such as drinking status and smoking status in a stratified analysis and

found that these environmental factors may interact with the analyzed SNPs.

Our study had several limitations. First, the research population was drawn only from the Guangxi Zhuang Autonomous Region. Whether the results apply to the Chinese population as a whole or to other ethnic groups remains to be seen. Second, because our study used a case-control format, recall bias was difficult to avoid. However, we sought to minimize recall bias by choosing patients newly diagnosed with HCC. Finally, the functional influence of the examined SNPs and the potential mechanisms need to be determined in functional validation tests.

In conclusion, *MCM4* rs2305952 CC and *CHEK1* rs515255 TC, TT, TC/TT may decrease the risk of HCC and *KAT2B* rs17006625 GG may increase the risk of HCC. In addition, we observed an increased risk associated with *KAT2B* rs17006625 GG in HBsAg-negative patients. Furthermore, we also observed a decreased risk associated with *MCM4* rs2305952 CC in HBsAg-positive patients and in also non-drinking patients and non-smoking patients, and with *CHEK1* rs515255 TC, TT, TC/TT in HBsAg-negative patients and in also non-drinking patients. Our results suggest that the genetic variants in the cell cycle pathway genes affect the risk of HCC, however, further studies are needed to confirm the findings.

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## COMMENTS

### Background

The uncontrollable proliferation of cancer cells is a crucial mechanism in cancer development and progression. Previous studies have shown that polymorphisms of cell cycle pathway genes are associated with cancer. However, their relationship with hepatocellular carcinoma (HCC) is unclear.

### Research frontiers

Despite reports of an association between polymorphisms in cell cycle pathway genes and cancer risk, little is known about the relationship between these polymorphisms and HCC risk.

### Innovations and breakthroughs

This study enrolled 1127 cases newly diagnosed with HCC and 1200 non-tumor patients. It comprehensively investigated the relationship between 15 SNPs in 12 cell cycle pathway genes and HCC risk.

### Applications

Since individuals with the *KAT2B* rs17006625 GG genotype may have an increased risk of HCC, they should be carefully monitored to reduce the occurrence and development of HCC.

## Terminology

A single nucleotide polymorphism (SNP) is a variation in the genomic DNA sequence. SNPs in some genes may cause an increased or decreased risk of HCC.

## Peer-review

The manuscript is interesting and provides relevant information. The study is a descriptive paper analyzing the polymorphism in HCC in a wide number of patients. The analyses are consistent with the results and the conclusions asserted in the manuscript.

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