

Genetic Polymorphisms in CD35 Gene Contribute to the Susceptibility and Prognosis of Hepatocellular Carcinoma

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Abstract

CD35, an important molecule implicated in inflammation and immunity, has been reported to contribute to several cancers. However, very few studies have investigated the relationship between CD35 polymorphisms and hepatocellular carcinoma (HCC). This study was conducted to investigate the association of tag SNPs in CD35 gene with HCC susceptibility and postoperative recurrence, attempting to illuminate the interaction of gene-environment in HCC. A total of 1233 Chinese Han people were recruited in this study, including 647 healthy controls and 586 HCC cases. Six Tag SNPs (rs10494885, rs2296160, rs3737002, rs3849266, rs669117, rs7525160) of CD35 were selected using HaploView 4.2 program and were genotyped by matrix assisted laser desorption ionization time of flight mass spectrometry method (MALDI-TOF-MS). Overall, mutation genotypes CC/CG of CD35 rs7525160 significantly increased the risk of HCC. Through stratification analysis, CD35 rs7525160 CC/CG genotypes were found to increase HCC risk in younger than 65 years patients, and was closely related to the pathological type of poor prognosis of HCC. Cox proportional hazard ratio model analysis unraveled rs7525160 CC/CG genotype remained a significant independent risk factor for postoperative recurrence of HCC. In conclusion, CD35 rs7525160 polymorphism may contribute to susceptibility and prognosis of HCC in Chinese Han population.

Introduction

Hepatocellular carcinoma (HCC), which accounts for more than 80% of primary liver cancers worldwide, is estimated to be the fourth most common cause of cancer-related death and poses severe disease burden^{1,2}. In China, liver cancer has long been dominating all the malignancies³, and even constitutes over half of the worldwide liver cancer cases (446,100 cases) and deaths (422,100 cases) in 2015⁴. A multitude of risk factors have been found to be associated with liver cancer, including viral factors (HBV and HCV) and non-viral factors, such as alcohol, smoking, aflatoxin, obesity, diabetes and non-alcoholic fatty liver disease et al⁵⁻⁹. However, those known risk factors do not fully explain the overall incidence of HCC. A conglomeration of studies has pinpointed many a genetic factor that is correlated with HCC susceptibility¹⁰⁻¹³. These evidences indicate the consequential role of genetic background when untangling HCC etiology. Therefore, it's imperative to better understand the molecular mechanisms of the initiation and progression of HCC for earlier diagnosis and efficacious therapeutic strategies¹⁴.

The complement system has been primarily viewed as the "first line of defense" against microbial intruders, and participates in diverse processes, such as clearance of immune complex, tissue regeneration, and lipid metabolism et al¹⁵. Meanwhile, increasing studies indicate that complement can promote tumor development in different ways, including sustained cellular proliferation, decreased apoptosis, enhanced invasion and metastasis et al¹⁶⁻¹⁸. Liver is the predominant organ for the synthesis of complement¹⁹, which in turn impacts on a variety of liver diseases, such as liver damage, regeneration and liver transplantation²⁰.

Complement regulatory proteins are a big family, which are essential for the activation of the complement system²¹. One of its family member named complement receptor 1 (CR1/CD35, hereafter named CD35), is strikingly significant because it interacts not only with C3b and C4b to promote neutrophil-mediated phagocytosis but also involved in T-cell and B-cell mediated immune regulation^{22,23}. Considering the important role of CD35 in complement activation, innate immunity and chronic inflammation, CD35 has become a hot molecule in the study of cancer predisposition, which has already been attested to by a few studies in gallbladder cancer, nasopharyngeal carcinoma, non-small cell lung cancer and gastric cancer et al²⁴⁻²⁷.

However, very few studies have investigated the relationship between CD35 polymorphisms and HCC predisposition, so we conduct this case-control study in order to investigate the association of tag SNPs in CD35 gene with susceptibility and recurrence of HCC, in an attempt to explore the interaction of gene-environment on the risk of HCC.

Materials And Methods

This study strictly followed the relevant Chinese laws, regulations and rules, the principles of the Declaration of Helsinki of the World Medical Association, and was approved by the Ethics Committee of Sichuan University (Ethics No. 2017 - 264).

Study population

Newly diagnosed, untreated HCC patients admitted to the Department of Liver Surgery of the West China Hospital in Sichuan University (Chengdu, China) from May 2017 to March 2019 were enrolled in this study. The diagnosis of HCC was based on histologically findings or the combination of an elevating serum α -fetoprotein (AFP) and the coincident imaging manifestations in two different imagological diagnosis (ultrasonography, computed tomography or magnetic resonance imaging). Staging criteria for liver cancer was based on Barcelona-Clinic-Liver-Cancer (BCLC) stages²⁸ and the TNM (tumor-node-metastasis) classification system²⁹. None of the patients received radiotherapy or anticancer cytotoxic drug chemotherapy 1 month before blood collection. Patients with previous malignancy or metastasized cancer in other organs, autoimmune diseases, Alzheimer's disease (AD) and HCC combined pregnancy were excluded. The healthy controls were those who underwent laparoscopic cholecystectomy for gallstone in the West China Hospital during the same period, and had no history of other diseases, tumors, autoimmune diseases or Alzheimer's disease. All subjects were genetic unrelated Han Chinese and there were no age or gender restrictions.

A written informed consent was obtained from each subject at recruitment. The baseline characteristics of each subject, such as age, gender, smoking history, drinking history, and family history of HCC, were obtained from the subjects or their family members, and the relevant information of HCC patients was further improved by referring to medical records, laboratory tests and imaging studies, such as tumor size, number of tumors, metastasis, Child-Pugh classification, microvascular invasion.

Selection of Tag SNPs and genotype analysis

All SNP genotyping of the CD35 gene was obtained and downloaded from the Human Genome Project and the International HapMap Project. Then HaploView 4.2 program was used to select the candidate tag SNPs of CD35 with an r^2 threshold of 0.8 and minor allele frequency (MAF) greater than or equal to 0.05 based on the Chinese population data from HapMap database. Finally, six SNPs (rs10494885, rs2296160, rs3737002, rs3849266, rs669117, rs7525160) were included in this study (supplementary table S1).

Genomic DNA was extracted from whole blood samples of EDTA anticoagulant using TIANamp Genomic DNA Kit (TIANGEN BIOTECH CO.LTD, BEIJING, CHINA) strictly according to the manufacturer's instructions. All extracted DNA specimens were diluted to 20 ng/ μ L for working concentration and stored at -20°C.

Genotyping was carried out by MassARRAY system (Sequenom, San Diego, CA, USA) using matrix assisted laser desorption ionization-time of flight mass spectrometry method (MALDI-TOF) according to the manufacturer's instructions. The detailed process for genotyping could be found in another paper of our team¹². Supplementary table S2 shows the primer information for selected tag SNPs. Genotyping quality control consisted of verifying genotypic consistency in 5% randomly selected duplicated samples and about 5% of the samples were further confirmed by direct sequencing.

HCC surveillance and follow-up

All HCC patients with hepatectomy treatments were regularly followed up in the Outpatient Department of Liver Surgery of the West China Hospital in Sichuan University (Chengdu, China). During the first year after surgery, the patients were followed up every three months, and the patients with no recurrence at least 2 years after surgery were followed up every six months instead. Abdominal ultrasonography and blood tests, including liver function tests, and AFP levels were performed at each follow-up. Any subject with a positive finding of AFP or abnormal ultrasonography was conducted enhanced computed tomography (CT) or magnetic resonance imaging (MRI) of the upper abdomen immediately. Recurrence Free Survival (RFS) was defined as the time interval from the date of hepatectomy to the date when the patients found recurrence or the deadline for the last follow-up. The Overall Survival (OS) is defined as the time interval from the date of hepatectomy to the date of death or the last follow-up.

Statistical analysis

SPSS version 24.0 statistical software (IBM Corp., Armonk, NY, USA) was used in performing statistical analysis. All tests were two-side, and $P < 0.05$ was considered statistical significance. Each SNP frequency in controls was analyzed for deviation from the Hardy-Weinberg equilibrium (HWE) using the chi-squared goodness-of-fit test. Mann-Whitney U test (for continuous variables) and Chi-squared tests with Fisher's exact test (for categorical variables) were used to compare the differences in the distributions of demographic characteristics between HCC and control group. The association between HCC risk and CD35 tag SNPs was estimated as odds ratios (OR) and 95% confidence intervals (CI) using

an unconditional logistic regression model, adjusted by gender, HBV status, smoking and drinking status where it was appropriate. The relationship between RFS/OS and CD35 tag SNPs was analyzed using the univariate survival analysis and cox proportional hazard ratio model. Family history of liver cancer is defined as family members within three generations of the patient have liver cancer. Ever/current smoking is defined as continuous/cumulative smoking for 6 months or more in a lifetime, and at least a total of 100 cigarettes. Those who had consumed 12 standard drinking amounts (1 standard drinking amount is equal to 10g of alcohol, or about 350ml of regular beer, or about 148ml of wine, or about 45ml of 40% white wine) in the past 12 months were defined as ever/current drinkers³⁰.

Consent for publication

All authors have read the final version of the paper and approved this submission.

Results

Patient characteristics

600 HCC cases and 650 cancer-free controls were included in this study initially. Due to unsuccessful genotyping of 14 HCC cases and 3 controls, a total of 1233 participants consisting of 586 HCC patients (491 males and 95 females) and 647 controls (297 males and 350 females) were included in this study at last. The baseline characteristics of HCC cases and controls were summarized in Table 1. There were no significant differences in age (stratified by 65 years) and family history of liver cancer, while gender, hepatitis B, smoking and drinking status were significant statistically different between two groups ($P < 0.05$). A total of 299 HCC patients underwent hepatectomy treatment and were followed to December 2019, the maximum follow-up time was 30.7 months with a medium follow-up time 18.0 months, and the characteristics and clinical features of HCC patients treated with hepatectomy were summarized in supplementary table S3.

Table 1
Baseline characteristics of HCC patients and controls

Variable	HCC	controls	P-value
	(n = 586)	(n = 647)	
Age [n (%)]			0.092
<65 years	444(75.80)	516(79.80)	
≥ 65 years	142(24.20)	131(20.20)	
Gender [n (%)]	491(83.80)	297(45.90)	< 0.001*
Male	95(16.20)	350(54.10)	
Female			
Smoking status [n (%)]			< 0.001*
Never	275(46.90)	506(78.20)	
Ever/current	311(53.10)	141(21.80)	
Drinking status [n (%)]			< 0.001*
Never	326(55.60)	491(75.90)	
Ever/current	260(44.40)	156(24.10)	< 0.001*
Hepatitis B [n (%)]	442(75.40)	143(22.10)	
With	144(24.60)	504(77.90)	
without			
Family history of liver cancer [n (%)]			0.347
without	560(95.60)	625(96.60)	
with	26(4.40)	22(3.40)	
*P< 0.05, statically significant.			

Genotype frequency and effects of CD35 on HCC risk

The genotype distribution of CD35 in controls followed the predictions of Hardy-Weinberg equilibrium (supplementary table S1, $P > 0.05$). Among six SNPs of CD35, only the frequencies of GG, CG, and CC genotypes of rs7525160 in HCC and control groups were statistically significant ($P < 0.05$, Table 2). After adjusting for gender, smoking, Hepatitis B and drinking status, the risk of HCC was 1.46-times higher in individuals with at least one mutant allele C (CG + CC) than in individuals with the GG genotype [adjusted OR = 1.46, 95% CI (1.09–1.95), $P = 0.012$] (Table 2). No significant differences were seen in the distribution of rs10494885, rs2296160, rs3737002, rs3849266 and rs6691117 genotypes between the

HCC and control group ($P > 0.05$, Table 2). The three genotypes of rs7525160 polymorphism detected by MALDI-TOF and sequencing map for CD35 rs7525160 GG and CG genotypes were summarized in supplementary figure S1.

Table 2
SNPs of CD35 gene in patients with HCC and controls

SNP ID	Genotype	HCC n (%)	Control n (%)	Adjusted OR (95% CI) ^a	P value
rs10494885	AA	74(12.60)	90(13.90)	1.00(Reference)	0.493
	AG	251(42.80)	275(42.50)	1.159(0.76, 1.77)	0.575
	GG	261(44.50)	282(43.60)	1.131 (0.74, 1.74)	0.479
	GG/AG	512(87.40)	557(86.10)	1.156(0.77, 1.73)	
rs2296160	AA	77(13.10)	79(12.20)	1.00(Reference)	0.443
	AG	240(41.00)	302(46.70)	0.84(0.54, 1.31)	0.922
	GG	269(50.40)	265(41.00)	0.98(0.63, 1.52)	0.652
	GG/AG	509(86.90)	567(87.80)	0.91(0.60, 1.38)	
rs3737002	CC	261(44.50)	279(43.10)	1.00(Reference)	0.571
	CT	245(41.80)	281(43.40)	1.09(0.81, 1.47)	0.917
	TT	80(13.70)	87(13.40)	1.02(0.66, 1.58)	0.639
	TT/CT	325(55.50)	368(56.90)	1.07(0.81, 1.41)	
rs3849266	CC	262(44.70)	283(43.90)	1.00(Reference)	0.506
	CT	244(41.60)	275(42.60)	1.11(0.82, 1.49)	0.902
	TT	80(13.70)	87(13.50)	1.03 (0.67, 1.58)	0.570
	TT/CT	324(55.30)	362(56.10)	1.08(0.82, 1.43)	
rs6691117	AA	288(49.10)	342(52.90)	1.00(Reference)	0.619
	AG	232(39.60)	255(39.40)	0.93(0.69, 1.24)	0.343
	GG	66(11.30)	50(7.70)	1.27(0.77, 2.08)	0.933
	GG/AG	298(50.90)	305(47.10)	0.99(0.75, 1.30)	
rs7525160	GG	180(30.70)	258(39.90)	1.00(Reference)	0.047*
	CG	286(48.80)	296(45.70)	1.37(1.01, 1.86)	0.011*
	CC	120(20.50)	93(14.40)	1.69(1.13, 2.54)	0.012*
	CC/CG	406(69.30)	389(60.10)	1.46(1.09,1.95)	

^a adjusted for gender, smoking, Hepatitis B and drinking status. * $P < 0.05$, statically significant.

Stratified analysis of demographic characteristics and environmental factors

We stratified to analyze the polymorphism of CD35 rs7525160 and HCC risk, based on factors including demographic characteristics (gender, age) and environmental factors (Hepatitis B, family history of liver cancer, drinking and smoking status) (supplementary table S4). After stratified analysis, we found that CD35 rs7525160 CC/CG genotype increased the risk of HCC in younger than 65 years patients [P for interaction = 0.042, adjusted OR = 1.85, 95% CI (1.30–2.62), P = 0.001].

Genotype frequency and effects on tumor clinicopathological types

Further stratified analysis of the clinical characteristics of HCC patients revealed that CD35 rs7525160 CC/CG genotype could increase the risk of different clinicopathological types of HCC, especially among patients with AFP \geq 400ng/ml [adjusted OR = 1.94, 95% CI (1.24–3.04), P = 0.004], tumor size of >5cm [adjusted OR = 1.75, 95% CI (1.19–2.56), P = 0.004], TNM stage III/IV [adjusted OR = 1.64, 95% CI (1.07–2.52), P = 0.024], with a background cirrhosis [adjusted OR = 1.50, 95% CI (1.05–2.15), P = 0.026], with portal vein tumor thrombosis [adjusted OR = 2.30, 95% CI (1.24–4.27), P = 0.008] (supplementary table S5).

CD35 genetic variation on postoperative recurrence of HCC and overall survival analysis

As aforementioned, CD35 rs7525160 pertained to increased HCC risk especially in advanced stage or big tumors, which suggested its potential predictive value in prognosis. To evaluate this, we detected the six CD35 SNPs genotype effects on the recurrence rate and mean recurrence-free survival (MRFS) in 299 HCC patients who underwent curative hepatectomy. Patients who were treated with Transcatheter Arterial Chemoembolization (TACE), radiofrequency ablation and drug only were excluded in order to exclude the influence of different treatments on the prognosis of HCC. In the Kaplan-Meier analyses, our results showed that the MRFS was 17.82 months with 95% CI 16.104–19.544 months in CD35 rs7525160 CC/CG individuals, which was shorter than in individuals with the GG genotype (MRFS 22.05 months, 95% CI 19.819–24.289 months, P = 0.0073). (Fig. 1)

Considering that the recurrence of HCC is related to many clinical factors, we firstly conducted univariate survival analysis to find the possible factors affect the recurrence of HCC in hepatectomy patients. Our result showed that HBV DNA level $\geq 10^2$ IU/mL [HR = 1.77, 95% CI (1.26,2.47), P = 0.011], Child-Pugh Class B [HR = 2.55, 95% CI (1.24,5.22), P = 0.011], Child-Pugh Class C [HR = 6.56, 95% CI (1.60,26.84), P = 0.009], with microvascular invasion [HR = 1.68, 95% CI (1.18,2.38), P = 0.004], tumor BCLC stage B/C [HR = 2.17, 95% CI (1.52,3.08), P < 0.001], AFP level \geq 400 ng/mL [HR = 1.68, 95% CI (1.19,2.38), P = 0.003], tumor size >5cm [HR = 1.55, 95% CI (1.11,2.17), P = 0.014], multiple tumor number [HR = 1.62, 95% CI (1.10,2.39), P = 0.014], TNM tumor stage III/IV [HR = 2.47, 95% CI (1.73,3.52), P < 0.001] and with portal vein tumor

thrombosis [HR = 2.44, 95% CI (1.58,3.76), $P < 0.001$] were the possible factors associated with the recurrence of HCC (Table 3). Then, cox proportional hazard ratio model was used to analyze the effects of CD35 rs7525160 genotype on HCC recurrence in hepatectomy patients. As shown in Table 4 and Fig. 2, CD35 rs7525160 remained a significant independent risk factor for postoperative recurrence of HCC [adjusted HR = 1.64, 95% CI (1.10–2.45), $P = 0.015$]. In overall survival analysis, at a median follow-up of 18.0 months and maximum follow-up time of 30.7 months, 27 of 299 patients (9.0%) had died. The univariable analyses of overall survival are summarized in supplementary table S6. No statistical significance was found between the six Tag SNPs of CD35 and the overall survival.

Table 3 Univariate survival analysis of clinical factors associated with HCC recurrence

Variable	n	Recur [n (%)]	HR (95% CI)	P-value
Age				
<65 years	235	112(81.20%)	1.00	0.174
≥65 years	64	26(18.8%)	0.74(0.49,1.14)	
Gender				
Female	48	24(17.4%)	1.00	0.473
Male	251	114(82.6%)	0.85(0.55,1.32)	
Hepatitis B				
Without	61	26(18.8%)	1.00	0.571
With	238	112(81.2%)	1.13(0.74,1.73)	
Smoking status				
Never	138	62(44.9%)	1.00	0.650
Ever/current	161	76(55.1%)	1.08(0.77,1.51)	
Drinking status				
Never	166	77(55.8%)	1.00	0.857
Ever/current	133	61(44.2%)	1.03(0.74,1.44)	
HBV DNA level (IU/mL)				
<10 ²	168	67(48.6%)	1.00	0.011*
≥10 ²	130	71(51.4%)	1.77(1.26,2.47)	
Child-Pugh Class				
A	286	128(92.8%)	1.00	
B	11	8(5.8%)	2.55(1.24,5.22)	0.011*
C	2	2(1.4%)	6.56(1.60,26.84)	0.009*
Microvascular invasion				
Without	202	87(63.5%)	1.00	0.004*
With	92	50(36.5%)	1.68(1.18-2.38)	
Tumor stage (BCLC)				
0/A	228	91(65.9%)	1.00	<0.001*
B/C	71	47(34.1%)	2.17(1.52,3.08)	
α-fetoprotein level(ng/mL)				

<400	208	85(61.6%)	1.00	0.003*
≥400	91	53(38.4%)	1.68(1.19,2.38)	
Tumor size (cm)				
≤5cm	150	60(43.5%)	1.00	0.011*
≥5cm	149	78(56.5%)	1.55(1.11-2.17)	
Tumor number				
Single	244	104(75.4%)	1.00	0.014*
Multiple	55	34(24.6%)	1.62(1.10,2.39)	
Tumor stage (TNM)				
I/II	233	92(66.7%)	1.00	<0.001*
III/IV	66	46(33.3%)	2.47(1.73,3.52)	
Background cirrhosis				
Absent	88	33(23.9%)	1.00	0.166
Present	211	105(76.1%)	1.32(0.89,1.95)	
Portal vein tumor thrombosis				
No	265	113(81.9%)	1.00	<0.001*
Yes	34	25(18.1%)	2.44(1.58,3.76)	
Distant metastasis				
No	297	137(99.3%)	1.00	0.880
Yes	2	1(0.7%)	1.16(0.16,8.33)	

* $P < 0.05$, statically significant.

Table 4. Cox analysis of potential factors for recurrence analysis in patients with resected HCC

Variables	Crude HR (95% CI)	Adjusted HR (95% CI) ^a	P-value (Wald's test)
rs7525160			
GG	1.00	1.00	
CG/CC	1.67 (1.14,2.44)	1.64 (1.10,2.45)	0.015*
HBV DNA level (IU/mL)			
<10 ²	1.00	1.00	
>10 ²	1.77 (1.26,2.47)	1.45 (1.01,2.08)	0.044*
Child-Pugh Class (C vs. B/A)			
A	1.00	1.00	
B	2.55 (1.24,5.22)	2.49 (1.16,5.36)	0.020*
C	6.56 (1.6,26.84)	7.62 (1.74,33.44)	0.007*
Microvascular invasion			
without	1.00	1.00	
with	1.68 (1.18,2.38)	1.18 (0.80,1.75)	0.403
Tumor stage (BCLC)			
0/A	1.00	1.00	
B/C	2.17 (1.52,3.08)	0.9 (0.36,2.27)	0.825
α-fetoprotein level (ng/mL)			
<400	1.00	1.00	
≥400	1.68 (1.19,2.38)	1.34 (0.92,1.95)	0.125
Tumor size (cm)			
≤5	1.00	1.00	
>5	1.55 (1.11,2.17)	1.05 (0.71,1.54)	0.806
Tumor number			
single	1.00	1.00	
multiple	1.62 (1.1,2.39)	1.48 (0.82,2.67)	0.195
Tumor stage (TNM)			
I/II	1.00	1.00	
III/IV	2.47 (1.73,3.52)	1.52 (0.69,3.37)	0.301
Portal vein tumor thrombosis			

without	1.00	1.00	
with	2.44 (1.58,3.76)	1.38 (0.66,2.88)	0.388

* $P < 0.05$, statically significant.

Discussion

Chronic inflammation is one of the hallmarks of cancer³¹, while the complement cascade, implicating in the immune and inflammatory responses, plays a pivotal role in tumorigenesis²⁴. CD35, as an important component of innate immunity, not only plays a key role in the clearance of circulating immune complexes³², but also inhibits the activity of C3 and C5 convertases in classical and alternative pathways and promotes their degradation, so as to regulate the complement cascade activation and inhibit the inflammatory response. Therefore, it is reasonable to postulate that the genetic variants of CD35 in the complement system confers susceptibility to cancer.

Indeed, quite a few studies have confirmed that the CD35 gene polymorphism is closely related to a variety of malignant tumors, such as nasopharyngeal carcinoma, gallbladder cancer, bladder cancer, non-Hodgkin's lymphoma, small cell lung cancer, stomach cancer, ovarian cancer^{24-26, 33}. However, scarce studies have investigated the relationship between CD35 polymorphisms and HCC. In our study, we have for the first time demonstrated that one SNP (rs7525160) out of 6 tag SNPs of CD35 was associated with the risk of HCC in Chinese Han population. Notably, compared with the GG genotype, the CD35 rs7525160 CC/CG genotype was associated with an increased risk of developing HCC.

Genetic mutation caused diseases usually have a characteristic of relatively younger age at occurrence, which probably attribute to their vulnerability to the genetic effects rather than environmental exposure in young patients and has been demonstrated in a number of studies³⁴⁻³⁶, and the potential gene-environmental interaction influences the susceptibility to malignant tumors. Consistent with their data, our results also showed that CD35 rs7525160 CC/CG genotype presented a risk factor for HCC in younger than 65 years patients.

With the popularization of screening among high-risk groups and the improvement of treatment regimens, the recurrence rate and fatality rate of HCC have decreased⁷, but remains by far the second most common cause of cancer-related death worldwide³⁷. Numerous predictors related to the prognosis of liver cancer have also been reported, such as AFP levels, tumor number and size³⁸, but no effective indicators have been found so far. Currently, studies have shown that SNPs can be used to predict the prognosis of many cancers³⁹. And our further subgroup analysis found that allele C of CD35 rs7525160 genotype frequency in HCC patients was significantly different from controls, especially in patients with AFP ≥ 400 ng/ml, tumor diameter of > 5 cm, TNM stage III/IV, cirrhosis, and portal vein thrombosis, which suggested that CD rs7525160 CC/CG genotype may be related to the type of HCC with poor prognosis. In order to eliminate the effect of different treatments on the prognosis of HCC, we only included 299

untreated patients with hepatectomy to analyze the relationship between CD35 gene and relapse-free survival/ overall survival. We found that the CD35 rs7525160 CC/CG genotypes were significantly associated with shorter mean relapse-free survival of HCC patients, and rs7525160 was identified as an independent predictor of relapse-free survival for HCC patients by Cox analysis. However, no relationship between CD35 gene polymorphism and overall survival was found in this study, which may be due to that the overall survival of postoperative recurrence of HCC is affected by many factors, including the choice of treatment after recurrence, the economic status of patients, and so on.

Conclusion

In summary, we found that the CD35 gene rs7525160 may be associated with increased HCC susceptibility in Chinese Han population, especially in younger than 65 years individuals. In addition, our results indicated that the CD35 rs7525160 CC/CG genotype is closely related to the pathological type of poor prognosis of HCC, and was an independent risk factor for a shorter recurrence-free survival time after hepatectomy. Our results suggest that CD35 rs7525160 might be used as a biomarker for screening and prognostic prediction of HCC. Additional well-designed studies with large sample size and long-term follow-up, encompassing different ethnic groups might be needed to better reveal the causal relationship between CD35 genetic polymorphism and HCC risk and prognosis.

Declarations

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Author contributions

L.-M. L. and Q.L. performed the research and wrote the paper. L.-M.L. and Z.-Z.S. were in charge of study conceptualization, investigation and validation. Q.L. and Z.-Z.S. analyzed the data. L.-X.L. and B.C. contributed the sample collection. Y.-F.P. and Y.-J.B. contributed the review and editing. F.L. was in charge of supervision, conceptualization and project administration. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

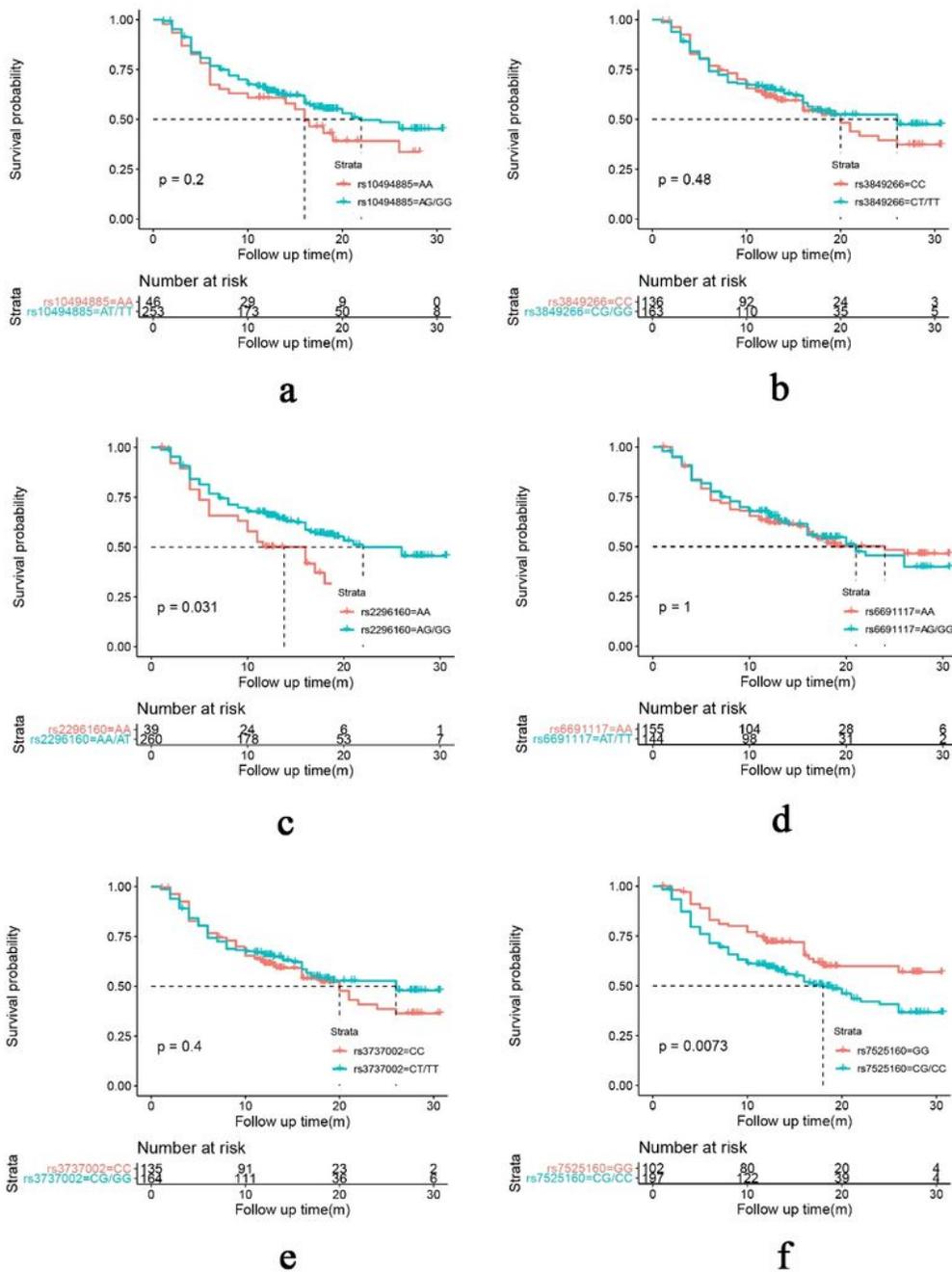


Figure 1

Kaplan-Meier analyses of CD35 genetic variation on postoperative HCC recurrence. (a) CD35 rs10494885. (b) CD35 rs3849266 (c) CD35 rs2296160. (d). CD35 rs6691117. (e) CD35 rs3737002 (f) CD35 rs7525160.

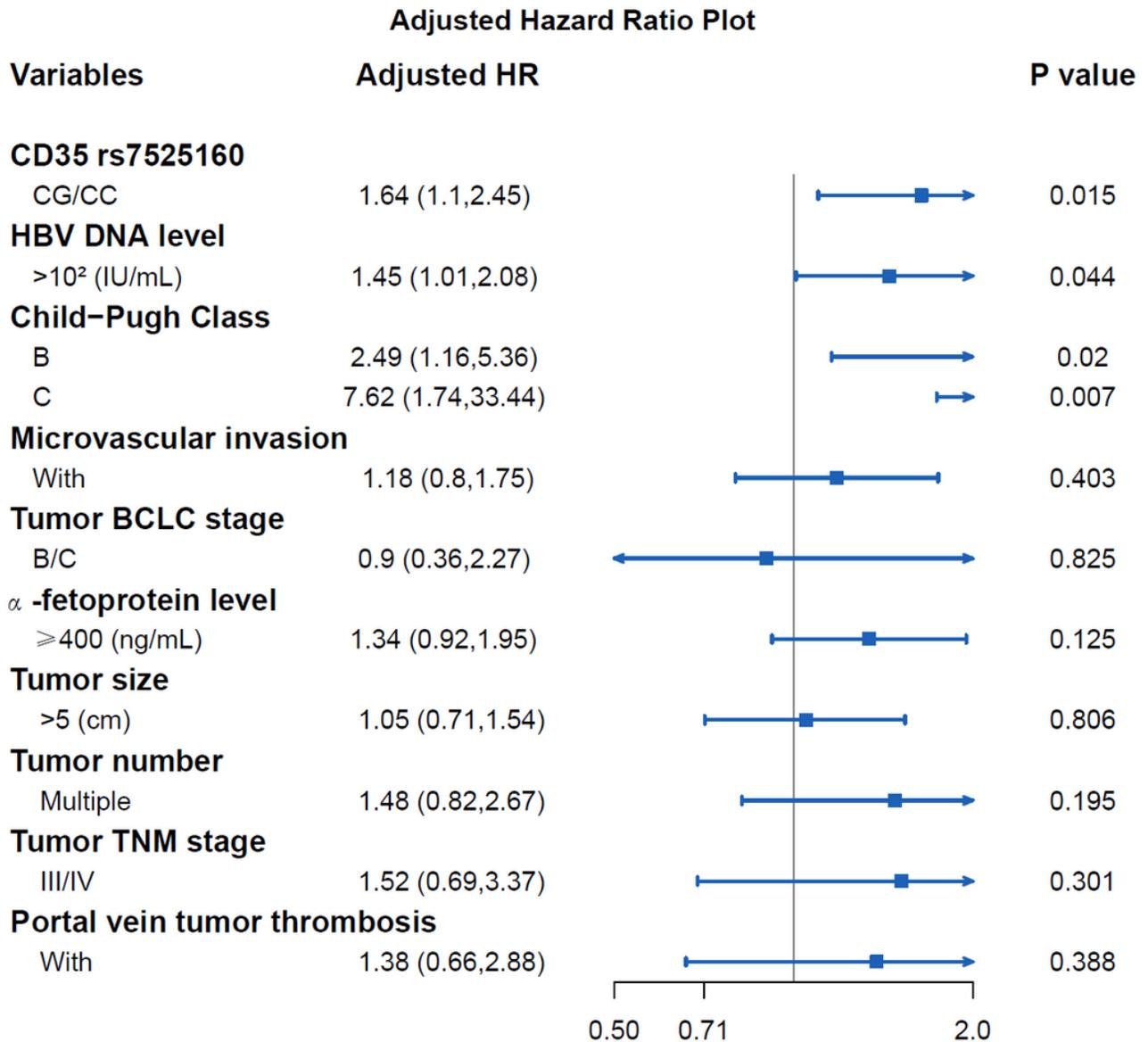


Figure 2

Cox analysis of potential factors for recurrence analysis in patients with resected HCC.

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