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ORIGINAL PAPER

A genetic variant (rs17251221) in the calcium-sensing receptor relates to hepatocellular carcinoma susceptibility and clinical outcome treated by transcatheter hepatic arterial chemoembolization (TACE) therapy

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Abstract Experimental and epidemiologic studies indicated that calcium-sensing receptor (CaSR) polymorphisms were associated with cancer risk, yet no data are available for candidate gene and hepatocellular carcinoma (HCC) risk. To address this, we evaluated whether *CaSR* rs17251221 polymorphism is associated with HCC susceptibility, clinicopathological parameters, and prognosis in HCC patients treated by TACE. A TaqMan assay was used to genotype rs17251221 SNP in this case ($n = 843$)–control ($n = 783$) study. A significant increased risk of HCC was observed in patients carrying rs17251221 GG (adjusted OR 1.355, 95 % CI 1.024–1.793, $P = 0.033$), AG/GG genotype (adjusted OR 1.254, 95 % CI 1.007–1.561, $P = 0.043$), and G allele (adjusted OR 1.163, 95 % CI 1.013–1.335, $P = 0.032$). Furthermore, a significant association was found between Child-Pugh class, serum BCLC stage, and AFP level and rs17251221 genotypes. More importantly, individuals carrying rs17251221 AG, GG genotype showed significantly longer MST than AA genotype and significant hazard ration (AG: adjusted HR 0.484, 95 % CI 0.406–0.577, $P < 0.001$; GG: adjusted HR 0.633, 95 % CI 0.575–0.697, $P < 0.001$, respectively). Meanwhile, we found a favorable HR for AG/GG genotype carriers (adjusted HR 0.645, 95 % CI 0.542–0.768, $P < 0.001$). These results indicated that *CaSR* rs17251221 polymorphism is associated with susceptibility to HCC, and rs17251221 G allele genotype showed significant independent better prognosis of HCC patients treated with TACE.

Keywords *CaSR* · Polymorphisms · Hepatocellular carcinoma · Susceptibility · Prognosis

Abbreviations

CaSR	Calcium-sensing receptor
SNPs	Single-nucleotide polymorphisms
HCC	Hepatocellular carcinoma
HBV	Hepatitis B virus
HBsAg	Hepatitis B surface antigen
AFP	Alpha fetoprotein
MST	Median survival time
DFS	Disease-free survival
OS	Overall survival
FEM	Regimen, 5-fluorouracil, epirubicin, and mitomycin-C
TACE	Transhepatic arterial chemotherapy and embolization
OR	Odds ratio

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CI Confidence interval
HR Hazard ratio

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death and is the fifth most common malignant tumor that accounts for ~80 % of all liver cancer cases worldwide [1, 2]. Etiologically, hepatocarcinogenesis is a multistep, multifactor, complex process [3]. Currently, the multiple risk factors mainly included hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, cirrhosis, carcinogen exposure, and especially genetic factors [4, 5]. Thus, substantial efforts should been made to identify genetic biomarkers as prognostic factors for improving therapeutic effect and prognosis prediction of HCC patients.

Calcium-sensing receptor (CaSR) was originally cloned from the bovine parathyroid gland in 1993 [6]. Subsequently, Canaff et al. [7] identified its expression in liver tissue and primary cultured hepatocytes. *CASR* gene located on chromosome 3p13.3–21, and encoded protein is a typical G protein coupled receptor with an extracellular domain, include a domain of seven membrane spanning and an unusually long intracellular carboxyl terminal tail [8, 9]. Generally, CaSR plays an important role in calcium homeostasis through regulation of parathyroid hormone secretion and calcium reabsorption [10, 11]. The rs17251221 SNP is located in the intron of the *CaSR* gene, and previous study revealed that rs17251221 polymorphism was strongly associated with serum calcium regulation in individuals of European and Indian-Asian descent [12]. Moreover, Chou et al. [13] revealed that the rs17251221 polymorphism was a susceptibility marker for stone multiplicity in nephrolithiasis. To data, only two studies investigated the association of *CaSR* rs17251221 genotypes with the susceptibility and survival of malignancies [14, 15]. One of the report found that *CaSR* (rs17251221) was strongly associated with prostate cancer [14]. Recent study further demonstrated that the genetic variations in the intron of *CaSR* gene rs17251221, a risk factor associated with breast cancer susceptibility, as well as a prognostic indicator [15]. However, the genetic effect of *CaSR* rs17251221 polymorphism on the susceptibility and prognosis of HCC is still unclear. Therefore, in this large prospective cohort, we analyzed the functional genetic polymorphisms of *CaSR* rs17251221 and attempted to elucidate the association between the polymorphisms and HCC susceptibility and clinical outcomes after FEM regimens treated with TACE. This study provides a theoretic basis for personalized chemotherapy in the treatment of HCC patients.

Materials and methods

Study subjects

We recruited 843 HCC patients newly diagnosed by cancer specialists consecutively from Shengjing Hospital of China Medical University and First Hospital of Dalian Medical University. This cohort of HCC patients was diagnosed based on histologic, combined with at least one positive HCC image on computed tomography (CT) or magnetic resonance imaging (MRI) and combined with serum AFP analysis (>400 ng/mL), and all HCC patients were untreated and shown not to have other cancers by an initial screening examination.

In this study, HCC typically are followed and monitored through their treatment regularly scheduled clinical and radiographic examinations. Preoperative serum tumor markers level included alpha fetoprotein (AFP). Clinical data were collected from medical records including sex, age, serum AFP, HBsAg marker, cirrhosis, tumor size, Child-Pugh class, TNM stage, and BCLC stage. All the patients ($n = 843$) received FEM (5-fluorouracil, epirubicin, mitomycin-C) regimen by transcatheter hepatic arterial chemoembolization (TACE) therapy. The FEM regimen consists of 5-fluorouracil (330 mg/m^2, every week), adriamycin (30 mg/m^2, every 4 weeks), and mitomycin (30 mg/m^2, every 2 weeks). The treatment was given or switched to other chemotherapy until disease progression, or patient's refusal to continue treatment.

We also included 783 unrelated healthy controls matched by gender and age. The controls had no known medical illness or hereditary disorders and were not taking any medications. The controls were recruited from individuals who visited the same hospitals for a health check-up. Before its commencement, this study was approved by the Research Ethics Committee of China Medical University and Dalian Medical University, and informed consent was obtained from each participant.

Genotyping assay

Genomic DNA was extracted from a leukocyte cell pellet of each blood sample using the TIANGEN DNA Blood Mini Kit (TIANGEN BIOTECH (Beijing) CO., LTD, Beijing, China) according to the manufacturer's instructions. *CaSR* rs17251221 polymorphism was genotyped by TaqMan SNP Genotyping Assays on the ABI 7500 fast Real-Time PCR platform (Applied Biosystems, ABI Technologies, Carlsbad, CA, USA). The TaqMan probes were synthesized by Life Technology (Shanghai, China). The following amplification conditions were used: 95 °C for 10 min, 45 cycles of 92 °C for 15 s, 60 °C for 60 s, and 60 °C for 30 s. Samples and 10 % duplicate *s* were

randomized and blinded for both cases and controls. There was 100 % concordance for both interplate and intraplate duplicates for rs17251221.

Statistical analysis

SPSS software package version 16.0 (SPSS Inc., Chicago, Illinois, USA) was used to analyze the data in this study. All tests were two-sided, and the criteria of statistical significance were set at $P < 0.05$. The chi-square (Pearson's χ^2 test) or Fisher's exact test was used to determine the differences in distributions of demographic, epidemiologic, and clinical variables between groups, and Hardy–Weinberg equilibrium test. Unconditional logistic regression models were used to analyze the association between the rs17251221 genotypes and HCC susceptibility, as well as clinicopathological parameters. The Disease-free survival (DFS) was taken from the date of diagnosis to the date of disease recurrence, progression, death without progression, or last follow-up. The overall survival (OS) was calculated as the time from diagnosis to liver cancer-related death or the date of last follow-up. The Kaplan–Meier method and the log-rank test were used to analyze the associations of the survival time with demographic characteristics, clinical features, and polymorphism. Multivariate Cox proportional hazards regression models were performed to obtain the adjusted hazard ratio (HR) and 95 % CI for potential prognostic factors for the survival in HCC patients.

Results

Demographic and baseline characteristics of the study subjects

The demographic characteristics and clinical variables of the HCC patients are summarized in Table 1. Totally, 843 patients with pathologically confirmed HCC and a group of 783 age- and gender-matched cancer-free healthy controls were included in this study. There were no significant differences in the distributions of gender and age between HCC patients and controls ($P = 0.693$ and $P = 0.732$, respectively). The age was matched between HCC patients (range 28–73 years; median 50 years) and controls (range 27–75 years; median 50 years). Among 843 patients, most of the patients were males (86.5 %), while 75.0 % of them had a chronic HBV infection. The vast majority of HCC patients (80.7 %) who had cirrhosis, and 69.8 % of the patients was classified into Child-Pugh A. All the HCC patients underwent the treatment of TACE. The variables of age, gender were adjusted for any residual confounding effects in later logistic regression analyses.

Table 1 Baseline variables in HCC patients ($n = 843$)

Characteristic	Total cohort	
	No.	%
Total no.	843	100
Median age [range], yrs	50 [22–85]	
Gender		
Male	729	86.5
Female	114	13.5
Age at diagnosis, yrs		
<50	345	40.9
≥50	498	59.1
Child-Pugh class		
A	588	69.8
B	255	30.2
BCLC class		
A	96	11.4
B	273	32.4
C	474	56.2
Tumor size (cm)		
≤5	386	45.8
>5	457	54.2
Clinical stage		
I or II	416	49.3
III or IV	427	50.7
HBV serological marker		
HBsAg (+)	632	75.0
HBsAg (+)	211	25.0
Cirrhosis		
Yes	680	80.7
No	163	19.3
Lymph node metastasis status		
Node-negative	705	83.6
Node-positive	138	16.4
Serum AFP level (ng/mL)		
≤400	474	56.2
>400	369	43.8
Therapeutic regimens		
FEM regimen treated with TACE	843	100

HBV hepatitis B virus, *HBsAg* hepatitis B surface antigen, *AFP* alpha fetoprotein, *FEM* regimen, 5-fluorouracil, epirubicin, and mitomycin-C, *TACE* transhepatic arterial chemotherapy and embolization

Association of *CaSR* rs17251221 polymorphism and HCC risk

The frequencies of allelic and genotype distribution for rs17251221 genetic variant in *CaSR* gene for both HCC patients and controls are shown in Table 2. The rs17251221 genotype frequency distribution in control group fit well to Hardy–Weinberg equilibrium ($P = 0.738$). We observed

Table 2 Frequency distribution of *CaSR* rs17251221 genotypes and their associations with the risk of developing HCC

Genotypes	Cases ($n = 843$) No. (%)	Controls† ($n = 783$) No. (%)	P‡	Adjusted OR (95 % CI)‡
CaSR rs17251221				
AA	198 (23.5)	160 (20.4)		1 (reference)
AG	435 (51.6)	393 (50.2)	0.103	1.212 (0.962–1.528)
GG	210 (24.9)	230 (29.4)	**0.033**	**1.355 (1.024–1.793)**
Dominant model				
GG	210 (24.9)	230 (29.4)		1 (reference)
GA/AA	633 (75.1)	553 (65.6)	0.137	0.837 (0.661–1.059)
Recessive model				
AA	198 (23.5)	160 (20.4)		1 (reference)
AG/GG	645 (76.5)	623 (79.6)	**0.043**	**1.254 (1.007–1.561)**
Allele frequency				
A allele	831 (49.3)	713 (45.5)		1 (reference)
G allele	855 (50.7)	853 (54.5)	**0.032**	**1.163 (1.013–1.335)**

Bold values indicate statistical significance ($P < 0.05$)

† The observed genotype frequency among individuals in the control group was in agreement with Hardy–Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$: $P = 0.738$ for *CaSR* rs17251221)

‡ P values and Adjusted OR and 95 % CI values were calculated by unconditional logistic regression adjusted for age, gender

that the alleles and genotypes from rs17251221 genetic variant were statistically associated with the risk of HCC. There was statistically increased risk of HCC in the homozygote comparison (GG vs. AA: adjusted OR 1.355, 95 % CI 1.024–1.793, $P = 0.033$), recessive model (AG/GG vs. AA: adjusted OR 1.254, 95 % CI 1.007–1.561, $P = 0.043$), and allele comparison (G vs. A: adjusted OR 1.163, 95 % CI 1.013–1.335, $P = 0.032$). No significant difference was detected for HCC risk in heterozygote comparison (GA vs. AA: adjusted OR = 0.894, 95 % CI = 0.697–1.147, $P = 0.379$) and dominant model (GA/AA vs. GG: adjusted OR 0.837, 95 % CI 0.661–1.059, $P = 0.137$).

Interaction of *CaSR* rs17251221 polymorphism with different clinical parameters in HCC patients

In case-only analysis ($n = 843$), we further investigated the interaction of *CaSR* rs17251221 polymorphism with clinicopathological characteristics or environmental risk factors such as HBsAg status, Cirrhosis using χ^2 test, and adjusted unconditional logistic regression adjusted by age and gender, shown in Table 3. We found that the distribution frequency of *CaSR* rs17251221 polymorphism was significantly associated with Child-Pugh class, BCLC stage, and serum AFP level. The frequency (81.2 %) of rs17251221 variants (AG/GG genotypes) in HCC patients with Child-Pugh class B was significantly higher than that (74.5 %) in those with Child-Pugh class A (adjusted OR 1.457, 95 % CI 1.010–2.100, $P = 0.044$), as shown in Table 3 and Fig. 1a. Furthermore, a higher frequency of rs17251221 AA genotype was observed in HCC patients with BCLC stage C (26.6 %) in comparison with those patients with BCLC stage A (67.5 %) (adjusted OR 0.389, 95 % CI 0.205–0.738, $P = 0.004$) (Table 3, Fig. 1b). There was a higher distribution frequency (26.8 %) of rs17251221 AA genotype was observed in serum AFP level (>400 ng/mL) than those HCC patients with lower serum AFP level (≤400 ng/mL) (adjusted OR 0.721, 95 % CI 0.523–0.995, $P = 0.046$) (Table 3; Fig. 1d). Moreover, the frequency distribution of rs17251221 genotypes in patients with different tumor size (cm) was shown in Fig. 1c, however, there was no association detected between the rs17251221 and tumor size (>5 vs. ≤5 cm) (adjusted OR 1.284, 95 % CI 0.930–1.772, $P = 0.128$). No significant interaction of rs17251221 polymorphism was found with other clinical characteristics.

Effects of *CaSR* rs17251221 polymorphism on HCC survival

Kaplan–Meier method and log-rank test, multivariate Cox regression analysis were performed to further evaluate the correlations of *CaSR* rs17251221 polymorphism with the prognosis of HCC patients treated with FEM regimen by TACE therapy ($n = 843$).

As shown in Fig. 2, a significant difference was observed between the OS of HCC patients and genotypes of rs17251221 (log-rank test: $P < 0.001$ in the different genotype and $P < 0.001$ in the recessive model, respectively). HCC patients carrying rs17251221 AG or GG genotype survived significantly longer OS time (AG genotype: median survival time, MST 22 months, 95 % CI 19–25 months, GG genotype: MST 31 months, 95 % CI 26–36 months, respectively) in comparison to the carriers who had AA genotype (MST 12 months, 95 % CI 8–14 months), as illustrated in Fig. 2a. Furthermore, multivariate Cox regression analysis also established that rs17251221 AG, GG genotypes as prognostic factors for longer OS time (AG genotype: adjusted HR 0.484, 95 % CI 0.406–0.577, $P < 0.001$; GG genotype: adjusted HR 0.633, 95 % CI 0.575–0.697, $P < 0.001$, respectively) adjusted by age, gender, child-Pugh class, tumor size, clinical stage, lymph node metastasis, serum tumor marker AFP level, outlined in Table 4. Then, in the recessive model, rs17251221 AG/GG genotype carriers (MST 27 months, 95 % CI 24–30 months; Fig. 2b) showed significantly prolonged OS time and also verified in multivariate Cox regression analysis (adjusted HR 0.645, 95 % CI 0.542–0.768, $P < 0.001$), as illustrated in Table 4. Moreover, after adjusted multivariate Cox regression analysis, we found that rs17251221 GG genotype

Table 3 Association between *CaSR* rs17251221 genotypes and clinicopathological features in HCC patients

Characteristic	CaSR rs17251221			
	AA No. (%)	AG/GG No. (%)	$P^{\dagger, \ddagger}$	Adjusted OR (95 % CI) [§]
Gender				
Male	174 (23.9)	555 (76.1)	0.510[†]	1 (reference)
Female	24 (21.1)	90 (78.9)	0.517[‡]	1.173 (0.724–1.898)
Age at diagnosis, yrs				
<50	87 (25.2)	258 (74.8)	0.324[†]	1 (reference)
≥50	111 (22.3)	387 (77.7)	0.328[‡]	1.174 (0.851–1.620)
Child-Pugh class				
A	150 (25.5)	438 (74.5)	**0.035**[†]	1 (reference)
B	48 (18.8)	207 (81.2)	**0.044**[‡]	**1.457 (1.010–2.100)**
BCLC stage				
A	12 (12.5)	84 (87.5)	**0.009**[†]	1 (reference)
B	60 (22.0)	213 (78.0)	0.145[‡]	0.769 (0.540–1.095)
C	126 (26.6)	348 (73.4)	**0.004**[‡]	**0.389 (0.205–0.738)**
Tumor size (cm)				
≤5	99 (25.6)	287 (74.4)	0.174[†]	1 (reference)
>5	99 (21.7)	358 (78.3)	0.128[‡]	1.284 (0.930–1.772)
Clinical stages				
I or II	96 (23.1)	320 (76.9)	0.781[†]	1 (reference)
III or IV	102 (23.9)	325 (76.1)	0.861[‡]	0.972(0.705–1.338)
HBV serological marker				
HBsAg (+)	156 (23.8)	500 (76.2)	0.707[†]	1 (reference)
HBsAg (+)	42 (22.5)	145 (77.5)	0.931[‡]	1.018 (0.680–1.524)
Cirrhosis				
Yes	162 (23.8)	518 (76.2)	0.638[†]	1 (reference)
No	36 (22.1)	127 (77.9)	0.716[‡]	1.080 (0.715–1.631)
Lymph node metastasis status				
Node-negative	168 (23.8)	537 (76.2)	0.596[†]	1 (reference)
Node-positive	30 (21.7)	108 (78.3)	0.565[‡]	1.138 (0.732–1.770)
Serum AFP level (ng/mL)				
≤400	99 (20.9)	375 (79.1)	**0.043**[†]	1 (reference)
>400	99 (26.8)	270 (73.2)	**0.046**[‡]	**0.721 (0.523–0.995)**

Bold values indicate statistical significance ($P < 0.05$)

[†] P value was obtained from two-sided chi-square test

[‡] P value was obtained from multinomial logistic regression analysis adjusted for age, gender

[§] Adjusted OR and 95 % CI was obtained from binary logistic regression analysis adjusted for age, gender

showed predictive of better prognosis in DFS (adjusted HR 0.815, 95 % CI 0.668–0.995, $P = 0.045$), although there was no association in log-rank test. In addition, multivariate Cox regression analysis identified that tumor size (>5 cm), clinical stages (III or IV), lymph node metastasis status (Node-positive), and serum AFP level (>400 ng/mL) of HCC patients was found to be predictive of worse prognosis in PFS or OS (Table 4).

Discussion

The calcium-sensing receptor (CaSR), as an important regulator of calcium homeostasis, is expressed in all of the organs including liver [16, 17]. The inactivation or mutation of the *CaSR* gene usually leads to one of the several disorders of calcium metabolism [18–20]. Recent study further demonstrated that high dietary Ca^{2+} stimulate CaSR activation and could down-regulate cell proliferation, invasion, inhibit tumor development, and increase the chemotherapeutic sensitivity of cancer cells [21–24]. Furthermore, epidemiological study revealed the *CaSR* polymorphisms contribute to susceptibility for breast cancer [15]. Therefore, rs17251221 polymorphism may be a potential candidate functional SNP in HCC.

To further confirm the above hypothesis, we for the first time performed a case–control study to explore systematically the correlation of *CaSR* rs17251221 polymorphism with the susceptibility, clinicopathological

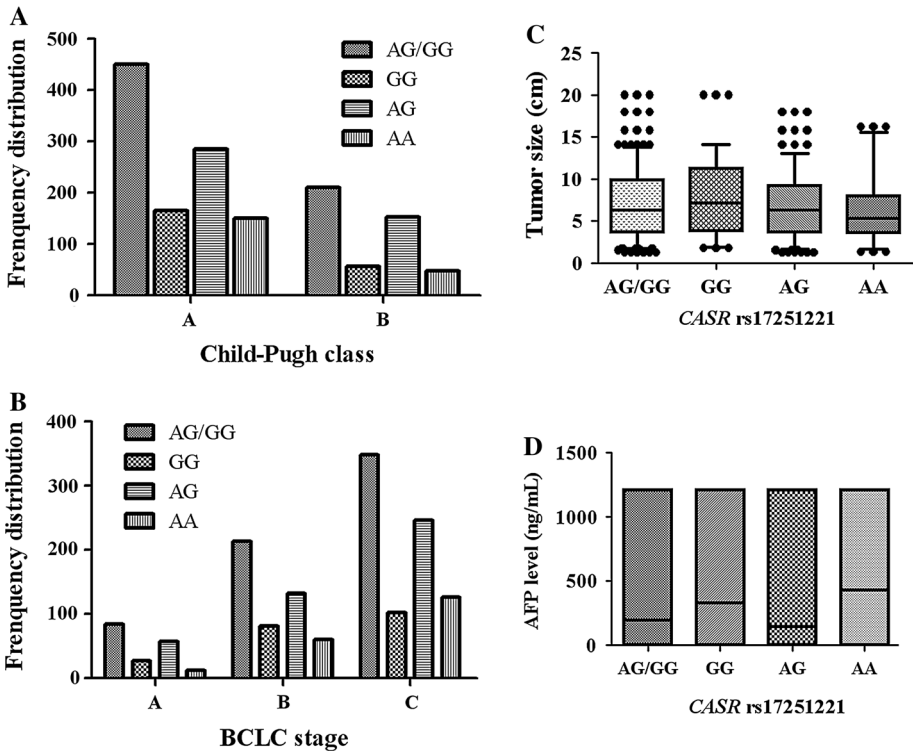

Fig. 1 Histogram and box plots illustrating the frequency distribution of rs17251221 genotypes and stratified clinicopathological characteristics. **a** Frequency distribution of rs17251221 genotypes classified by Child-Pugh classes (*a*, *b*). **b** Frequency distribution of rs17251221 genotypes classified by BCLC stage (*a*, *b*, *c*). **c** Mean tumor size (cm) (95 % CI) of HCC patients with rs17251221 genotypes (AG/GG, GG, AG, AA). **d** Median serum AFP level of HCC patients with rs17251221 genotypes (AG/GG, GG, AG, AA)

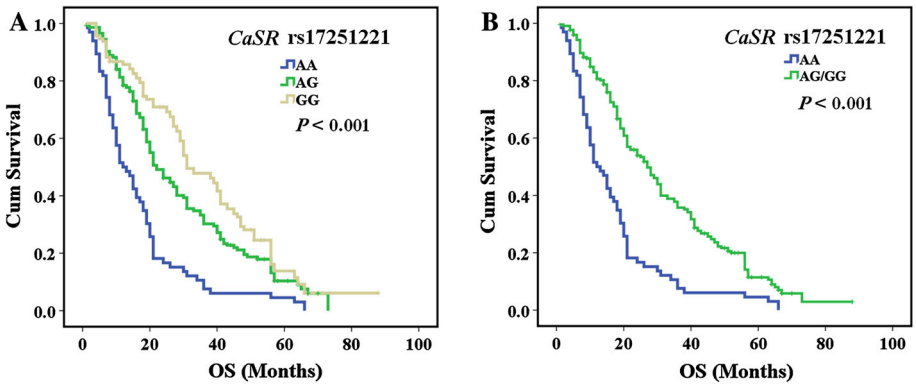

Fig. 2 The relationship between the *CaSR* rs17251221 polymorphism and HCC prognosis according to Kaplan–Meier analysis. **a** *CaSR* rs17251221 AG, GG genotype had longer overall survival in HCC patients after FEM regimens treated with TACE (log-rank test: $P < 0.001$). **b** *CaSR* rs17251221 AG/GG genotypes had longer overall survival in HCC patients after FEM regimens treated with TACE (log-rank test: $P < 0.001$)

parameters, and clinical outcomes of HCC patients after FEM regimen by TACE therapy. We found that rs17251221 AG/GG genotypes were associated with increased HCC susceptibility. These results are similar with the recent study [15] on breast cancer, reporting that patients with rs17251221 GG + AG genotypes were more likely to be increased risk for breast cancer than AA genotype carriers. More importantly, we further analyzed the relationship between rs17251221 polymorphism with clinicopathological parameters or environmental risk factors (HBsAg status, Cirrhosis status) and found that *CaSR* rs17251221 polymorphism was significantly associated with Child-Pugh class, BCLC stage, and serum AFP level, suggesting that rs17251221 polymorphism may be involved in the development of Child-Pugh class- and BCLC stage-associated HCC. This polymorphism may be used as a potential marker for identification of the malignant and invasive Child-Pugh class- and BCLC stage-associated HCC patients. However, we did not found any interaction with environmental risk factors such as HBsAg status and Cirrhosis status.

Table 4 Multivariate COX regression analysis of *CaSR* rs17251221 genetic polymorphisms and patient clinicopathological features in association with DFS and OS in HCC patients after FEM regimens treated with TACE

Variable	DFS				OS			
	Total N	Events N (%)	Adjusted HR (95 % CI)†	$P^{\dagger}$	Total N	Events N (%)	Adjusted HR (95 % CI)†	$P^{\dagger}$
CaSR rs17251221								
AA	198	27 (13.6)	1 (reference)		198	2 (1.0)	1 (reference)	
AG	435	111 (25.5)	0.963 (0.623–1.488)	0.865	435	63 (14.5)	**0.484 (0.406–0.577)**	**<0.001**
GG	210	57 (27.1)	**0.815 (0.668–0.995)**	**0.045**	210	48 (22.9)	**0.633 (0.575–0.697)**	**<0.001**
AG/GG	645	168 (26.1)	0.906 (0.598–1.374)	0.643	645	111 (17.2)	**0.645 (0.542–0.768)**	**<0.001**
Child-Pugh class								
A	588	138 (23.5)	1 (reference)		588	81 (13.8)	1 (reference)	
B	255	57 (22.4)	1.190 (0.867–1.632)	0.281	255	30 (11.8)	0.829 (0.534–1.287)	0.403
Tumor size (cm)								
≤5	386	99 (25.6)	1 (reference)		386	51 (13.2)	1 (reference)	
>5	457	96 (21.0)	**11.628 (6.944–19.608)**	**<0.001**	457	60 (13.1)	**4.049 (2.463–6.623)**	**<0.001**
Clinical stages								
I or II	416	93 (22.4)	1 (reference)		416	45 (10.8)	1 (reference)	
III or IV	427	102 (23.9)	**1.399 (1.186–1.653)**	**<0.001**	427	66 (15.5)	**1.748 (1.117–2.740)**	**0.014**
Lymph node metastasis status								
Node-negative	705	174 (24.7)	1 (reference)		705	90 (12.8)	1 (reference)	
Node-positive	138	21 (15.2)	**1.291 (1.055–1.579)**	**0.013**	138	21 (15.2)	1.213 (0.747–1.969)	0.435
Serum AFP level (ng/mL)								
≤400	474	129 (27.2)	1 (reference)		474	63 (13.3)	1 (reference)	
>400	369	66 (17.9)	**1.366 (1.168–1.597)**	**<0.001**	369	48 (13.0)	1.278 (0.863–1.892)	0.220

Bold values indicate statistical significance ($P < 0.05$)

† *P* value, Adjusted HR (95 % CI) were assessed using multivariate Cox regression analysis adjusted by age, gender, child-Pugh class, tumor size, clinical stage, lymph node metastasis, serum tumor marker AFP level

Little is known about regarding the *CaSR* rs17251221 polymorphism in terms of the potential impact on the cancer clinical outcomes. Thereafter, we further investigated the association of *CaSR* rs17251221 polymorphism with the survival time of HCC patients treated with TACE. It is worth to note that *CaSR* rs17251221 AG/GG genotypes were related to a significantly longer OS in our study, and multivariate analysis confirmed an independent favorable prognostic value of rs17251221 polymorphism in HCC patients treated by TACE. Similarly, in breast cancer [15], rs17251221 GG + AG genotypes were observed as prognostic indicators for both DFS and OS. However, further investigations are warranted to understand the precise mechanism of this polymorphism involved in different tumors in other areas or ethnicities.

To the best of our knowledge, our results firstly provide evidence that *CaSR* rs17251221 polymorphism is associated with susceptibility and therapeutic outcome in HCC patients treated with FEM chemotherapy by TACE therapy in a large and well-characterized cohort. These data suggest that *CaSR* rs17251221 polymorphism may play an important role in the development of HCC, and therefore, may be a vital prognostic indicator for HCC, and employed as a potential adjuvant in HCC patients for TACE therapy in the future.

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Conflict of interest None of the authors have any financial or other interests that could be construed as a conflict of interest with regard to the submitted manuscript.

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