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Research Paper

Impact of matrix metalloproteinase-11 gene polymorphisms upon the development and progression of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is a liver malignancy and a major cause of cancer mortality worldwide. Matrix metalloproteinase-11 (MMP-11), also known as stromelysin-3, plays a critical role during tumor migration, invasion and metastasis. Here, we report on the association between five single nucleotide polymorphisms (SNPs) – rs738791, rs2267029, rs738792, rs28382575, and rs131451 – of the *MMP-11* gene and HCC susceptibility, as well as clinical outcomes, in 293 patients with HCC and in 586 cancer-free controls. We found that carriers of the CT+TT allele of the rs738791 variant were at greater risk of HCC compared with wild-type (CC) carriers. Moreover, carriers of at least one C allele (C/T+C/C genotype) at the *MMP-11* SNP rs738792 were likely to progress to Child-Pugh B or C grade, while individuals with at least one C allele (C/T+C/C genotype) at the *MMP-11* SNP rs28382575 were at higher risk of developing stage III/IV disease, large tumors or lymph node metastasis. We believe that genetic variations in the *MMP-11* gene may help to predict early-stage HCC and act as reliable biomarkers for HCC progression.

Key words: *MMP-11* polymorphisms; Hepatocellular carcinoma; Single nucleotide polymorphism; Susceptibility

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer amongst men worldwide and the ninth in women, and a major cause of cancer-related mortality [1]. HCC is associated with a low 5-year survival rate and an increasing mortality rate [2, 3]. In Taiwan, HCC is the second leading cause of cancer-associated deaths [4, 5].

Genetic variation plays a key role in HCC susceptibility and development of the disease. The majority of people who are exposed to the well-known infectious, lifestyle or environmental risk factors (i.e., hepatitis B or C virus infection, alcohol abuse or non-alcoholic fatty liver disease associated with obesity, type 2 diabetes or insulin resistance) do not develop HCC, which suggests that individual susceptibility modulates tumorigenesis [4]. Genotype distribution frequency data can be used to map single nucleotide polymorphism (SNP) diversity in a

population and to examine the risk and development of specific diseases [6]. Emerging reports indicate an association between SNPs in certain genes and the susceptibility and clinicopathological status of HCC. For instance, individuals carrying specific interleukin-18 (*IL-18*), high-mobility group box protein 1 (*HMGB1*) or C-C chemokine ligand 4 (*CCL4*) SNPs are at higher risk of HCC than wild-type carriers [7-9].

Metastasis is a key step in tumor development and the chief cause of mortality for patients with cancer. There are several steps by which cells detach from the primary tumor and form a secondary tumor at a distant site [10]. Matrix metalloproteinases (MMPs) are well-known proteases associated with the breakdown of the extracellular matrix (ECM) surrounding tumor cells [11]. Increasing evidence has indicated that raised levels of MMPs are associated with cancer development and are linked to shorter survival of patients [12].

MMP-11, also known as stromelysin-3, has been observed during wound healing in normal physiologic conditions and in intense tissue remodeling during embryogenesis, as well as tissue involution [13]. However, MMP-11 is unlike most MMP family members which does not cleave major components of the ECM. In addition, the laminin receptor, insulin-like growth factor binding protein 1, collagen VI, and α1-proteinase inhibitor are major substrates of MMP-11 [14]. Upregulation of MMP-11 expression has been found in human carcinomas, such as lung, ovarian, breast, colorectal, and HCC [15, 16]. Genetic polymorphisms of *MMP-11* have been indicated in several different cancer types, including oral and breast cancers [17, 18]. Scant research has examined the association between *MMP-11* SNPs, HCC risk and prognosis. We therefore conducted a case-control study to evaluate the role of five *MMP-11* SNPs on HCC susceptibility and clinicopathological features in a cohort of Chinese Han individuals.

Materials and Methods

Participants

We enrolled 293 patients (cases) presenting with HCC to Chung Shan Medical University Hospital, Taiwan, between 2007 and 2015. A total of 586 anonymized healthy controls (HCs) without a history of cancer were randomly selected from the Taiwan Biobank Project. All study participants were of Chinese Han ethnicity. HCC patients were staged according to the 2010 American Joint Committee on Cancer (AJCC) TNM staging system, which incorporates tumor morphology, the number of lymph nodes affected, and metastases [19]. Before entering the study, each participant provided informed written consent and completed a structured questionnaire about sociodemographic status, cigarette and alcohol use. Liver cirrhosis was diagnosed by biopsy, appropriate sagittal CT or MRI scans, or biochemical evidence of liver parenchymal damage with endoscopic esophageal or gastric varices. The study was approved prior to commencement by Chung Shan Medical University Hospital's Institutional Review Board.

SNP selection

Five SNPs in *MMP-11* were selected from the International HapMap Project data for this study. We included the nonsynonymous SNPs rs738792 (Ala38Val) and synonymous SNPs rs28382575 (Pro475Pro) in the coding sequences of the gene. To obtain adequate power to evaluate the potential association, we investigated rs2267029 with minor allelic frequencies of >5%. Furthermore, the rs738791 and rs131451 were selected in this study because the gene polymorphism of the SNP has been found to associate with myopia [20].

Determination of genotypes

Total genomic DNA was isolated from whole blood specimens using QIAamp DNA blood mini kits (Qiagen, Valencia, CA), as per the manufacturer's instructions. This DNA was dissolved in TE buffer (10 mM Tris pH 7.8, 1 mM EDTA) and stored at −20°C until it was subjected to quantitative polymerase chain reaction (PCR) analysis. Five *MMP-11* SNPs (rs738791, rs2267029, rs738792, rs28382575, and rs131451) with minor allele frequencies >5% in the HapMap population were selected. The *MMP-11* SNPs were examined using the commercially available TaqMan SNP genotyping assay (Applied Biosystems, Warrington, UK), according to the manufacturer's protocols [21].

Bioinformatic analysis

Genotype-tissue expression (GTEx) data were used to identify correlations between SNPs and levels of *MMP-11* expression [22, 23]. We conducted an investigation into expression quantitative trait loci (eQTLs), to determine the functional role of phenotype-associated SNPs.

Statistical analysis

The genotype distribution of each SNP was analyzed for Hardy–Weinberg equilibrium and confirmed by Chi-square analysis. Demographic characteristics were compared between patients and controls using the Mann–Whitney U-test and Fisher's exact test. Associations between genotypes, HCC risk and clinicopathological characteristics were estimated using adjusted odds ratios (AORs) and 95%

confidence intervals (CIs) obtained from age- and gender-adjusted multiple logistic regression models. A p value of < 0.05 was considered statistically significant. Data were analyzed using SAS statistical software (Version 9.1, 2005; SAS Institute Inc., Cary, NC).

Results

Demographic characteristics did not differ significantly between the 293 patients with HCC and 586 cancer-free healthy controls (HCs) (Table 1). Significantly fewer (p < 0.001) controls compared with patients reported that they consumed alcohol, but cigarette smoking status did not differ between the two groups (p = 0.809) (Table 1). Compared with controls, significantly higher proportions of HCC patients were positive for hepatitis B surface antigen (HBsAg) (12.1% vs 42.3%; p < 0.001) and anti-hepatitis C virus (HCV) antibodies (4.4% vs 47.8%; p < 0.001) (Table 1). At study entry, 205 patients (70.0%) had stage I/II HCC and 88 (30.0%) had stage III/IV disease. Most patients had liver cirrhosis (83.3%) (Table 1).

Table 1. Demographic characteristics for 586 healthy volunteers (controls) and 293 patients with hepatocellular carcinoma.

Variable	Controls (N=586)	Patients (N=293)	p value
Age (yrs)	Mean ± S.D.	Mean ± S.D.	
	59.39 ± 7.45	60.08 ± 9.50	p = 0.284
Gender			
Male	426 (72.7%)	213 (72.7%)	
Female	160 (27.3%)	80 (27.3%)	p = 1.000
Cigarette smoking status			
No	347 (59.2%)	171 (58.4%)	
Yes	239 (40.8%)	122 (41.6%)	p = 0.809
Alcohol consumption			
No	501 (85.5%)	184 (62.8%)	
Yes	85 (14.5%)	109 (37.2%)	p < 0.001*
HBsAg			
Negative	515 (87.9%)	169 (57.7%)	
Positive	71 (12.1%)	124 (42.3%)	p < 0.001*
Anti-HCV			
Negative	560 (95.6%)	153 (52.2%)	
Positive	26 (4.4%)	140 (47.8%)	p < 0.001*
Stage			
I+II		205 (70.0%)	
III+IV		88 (30.0%)	
Tumor T status			
T1+T2		206 (70.3%)	
T3+T4		87 (29.7%)	
Lymph node status			
N0		283 (96.6%)	
N1+N2+N3		10 (3.4%)	
Metastasis			
M0		281 (95.9%)	
M1		12 (4.1%)	
Child-Pugh grade			
A		227 (77.5%)	
B or C		66 (22.5%)	
Liver cirrhosis			
Negative		49 (16.7%)	
Positive		244 (83.3%)	

Mann-Whitney U test or Fisher's exact test was used between healthy controls and patients with HCC.
* p value < 0.05 was considered statistically significant.

The distribution of the *MMP-11* genotypes between the HCC patients and HCs is shown in Table 2. In the HCs, all genotypic frequencies were in Hardy–Weinberg equilibrium (p > 0.05). In both patients and controls, most of those with the rs738791 SNP were homozygous for the C/C genotype, most of those with the rs2267029 SNP were homozygous for the G/G genotype, most of those with the rs738792 SNP were homozygous for T/T, most of those with the rs28382575 SNP were homozygous for T/T and most of those with the rs131451 SNP were homozygous for T/T (Table 2). After adjusting for potential confounders, subjects with CT+TT of the *MMP-11* rs738791 polymorphism had a 1.389-fold- (95% CI: 1.004-1.921; p < 0.05) higher risk of developing HCC compared to those with C/C homozygotes. However, there were no significant between-group differences as to the proportions of HCC patients with the rs2267029, rs738792, rs28382575 and rs131451 polymorphisms, as compared with HCs (Table 2).

Table 2. Genotyping and allele frequency of *MMP-11* single nucleotide polymorphisms (SNPs) in controls and patients with hepatocellular carcinoma.

Variable	Controls (N=586)	Patients (N=293)	Odds Ratio (95% Confidence Interval)[a]
rs738791			
CC	279 (47.6%)	121 (41.3%)	1.000 (reference)
CT	248 (42.3%)	143 (48.8%)	1.402 (0.999-1.967)
TT	59 (10.1%)	29 (9.9%)	1.332 (0.767-2.312)
CT+TT	307 (52.4%)	172 (58.7%)	1.389 (1.004-1.921)[b]
rs2267029			
GG	315 (53.8%)	155 (52.9%)	1.000 (reference)
AG	228 (38.9%)	116 (39.6%)	1.113 (0.798-1.551)
AA	43 (7.3%)	22 (7.5%)	0.970 (0.503-1.872)
AG+AA	271 (46.3%)	138 (47.1%)	1.092 (0.793-1.505)
rs738792			
TT	287 (49.0%)	149 (50.9%)	1.000 (reference)
CT	244 (41.6%)	119 (40.6%)	0.967 (0.693-1.348)
CC	55 (9.4%)	25 (8.5%)	0.770 (0.419-1.416)
CT+CC	299 (51.0%)	144 (49.1%)	0.933 (0.677-1.284)
rs28382575			
TT	562 (95.9%)	281 (95.9%)	1.000 (reference)
CT	23 (3.9%)	12 (4.1%)	0.654 (0.272-1.571)
CC	1 (0.2%)	0 (0.0%)	-
CT+CC	24 (4.1%)	12 (4.1%)	0.621 (0.260-1.483)
rs131451			
TT	198 (33.8%)	115 (39.3%)	1.000 (reference)
CT	278 (47.4%)	134 (45.7%)	0.907 (0.640-1.285)
CC	110 (18.8%)	44 (15.0%)	0.656 (0.400-1.077)
CT+CC	388 (66.2%)	178 (60.7%)	0.841 (0.603-1.172)

[a] adjusted for the effects of age and gender.
[b] p = 0.047.

Next, we compared the distributions of clinical aspects and *MMP-11* genotypes in HCC patients. Compared with patients with the T/T genotype, those with at least one polymorphic C allele at the rs738792 SNP (C/T+C/C genotype) were prone to developing moderate to severe liver failure (Child-Pugh B or C

grade; p = 0.008) (Table 3). Moreover, carriers of the C/T+C/C genotype of rs28382575 had a higher risk than T/T carriers of developing stage III/IV disease (p = 0.039), large tumors (p = 0.036) or lymph node metastasis (p = 0.001) (Table 4).

Table 3. Odds ratios (ORs) and 95% confidence intervals (CIs) associated with clinical status and *MMP-11* rs738792 genotypic frequencies in 293 patients with hepatocellular carcinoma.

Variable	Genotypic frequencies			
	TT (N=149)	CT+CC (N=144)	OR (95% CI)	*p* value
Clinical Stage				
Stage I/II	109 (73.2%)	96 (66.7%)	1.00	p = 0.227
Stage III/IV	40 (26.8%)	48 (33.3%)	1.362 (0.825-2.249)	
Tumor size				
≤T2	110 (73.8%)	96 (66.7%)	1.00	p = 0.181
>T2	39 (26.2%)	48 (33.3%)	1.410 (0.852-2.333)	
Lymph node metastasis				
No	146 (98.0%)	137 (95.1%)	1.00	p = 0.193
Yes	3 (2.0%)	7 (4.9%)	2.487 (0.630-9.809)	
Distant metastasis				
No	144 (96.6%)	137 (95.1%)	1.00	p = 0.518
Yes	5 (3.4%)	7 (4.9%)	1.471 (0.456-4.747)	
Vascular invasion				
No	126 (84.7%)	112 (77.8%)	1.00	p = 0.139
Yes	23 (15.4%)	32 (22.2%)	1.565 (0.865-2.832)	
Child-Pugh grade				
A	125 (83.9%)	102 (70.8%)	1.00	**p = 0.008***
B or C	24 (16.1%)	42 (29.2%)	2.145 (1.218-3.776)	
HBsAg				
Negative	82 (55.0%)	87 (60.4%)	1.00	p = 0.096
Positive	67 (45.0%)	57 (39.6%)	0.762 (0.554-1.049)	
Anti-HCV				
Negative	80 (53.7%)	73 (50.7%)	1.00	p = 0.647
Positive	69 (46.3%)	71 (49.3%)	0.924 (0.659-1.295)	
Liver cirrhosis				
Negative	26 (17.4%)	23 (16.0%)	1.00	p = 0.735
Positive	123 (82.6%)	121 (84.0%)	1.112 (0.601-2.056)	

The ORs with analyzed by their 95% CIs were estimated by logistic regression models.

> T2: multiple tumor more than 5 cm or tumor involving a major branch of the portal or hepatic vein(s)

* p value < 0.05 as statistically significant.

When we investigated associations between *MMP-11* gene polymorphisms and serum levels of alpha-fetoprotein (AFP), aspartate transaminase (AST) and alanine transaminase (ALT) in HCC patients [24], we found no significant associations between the levels of these HCC clinical pathologic markers and genotypes of any *MMP-11* SNPs (Table 5).

We searched the GTEx database to investigate whether rs738792 was associated with MMP-11 expression. Individuals carrying a genotype with the variant C at rs738792 showed a trend for reduced expression of MMP-11, compared with the wild-type TT homozygous genotypes (p < 0.05; Figure 1).

Table 4. Odds ratios (ORs) and 95% confidence intervals (CIs) associated with clinical status and *MMP-11* rs28382575 genotypic frequencies in 293 patients with hepatocellular carcinoma.

Variable	Genotypic frequencies			
	TT (N=281)	CT+CC (N=12)	OR (95% CI)	*p* value
Clinical Stage				
Stage I/II	200 (71.2%)	5 (41.7%)	1.00	**p = 0.039***
Stage III/IV	81 (28.8%)	7 (58.3%)	3.457 (1.066-11.208)	
Tumor size				
□ T2	201 (71.5%)	5 (41.7%)	1.00	**p = 0.036***
> T2	80 (28.5%)	7 (58.3%)	3.517 (1.085-11.407)	
Lymph node metastasis				
No	274 (97.5%)	9 (75.0%)	1.00	**p = 0.001***
Yes	7 (2.5%)	3 (25.0%)	13.048 (2.892-58.868)	
Distant metastasis				
No	269 (95.7%)	12 (4.3%)	1.00	-
Yes	12 (100.0%)	0 (3.2%)	-	
Vascular invasion				
No	230 (81.8%)	8 (66.7%)	1.00	p = 0.198
Yes	51 (18.2%)	4 (33.3%)	2.255 (0.654-7.776)	
Child-Pugh grade				
A	218 (77.6%)	9 (75.0%)	1.00	p = 0.834
B or C	63 (22.4%)	3 (25.0%)	1.153 (0.303-4.389)	
HBsAg				
Negative	162 (57.7%)	7 (58.3%)	1.00	p = 0.419
Positive	119 (42.3%)	5 (41.7%)	0.692 (0.284-1.688)	
Anti-HCV				
Negative	147 (52.3%)	6 (50.0%)	1.00	p = 0.930
Positive	134 (47.7%)	6 (50.0%)	1.039 (0.447-2.414)	
Liver cirrhosis				
Negative	46 (16.4%)	3 (25.0%)	1.00	p = 0.438
Positive	235 (83.6%)	9 (75.0%)	0.587 (0.153-2.252)	

The ORs with analyzed by their 95% CIs were estimated by logistic regression models.

> T2: multiple tumor more than 5 cm or tumor involving a major branch of the portal or hepatic vein(s)

* p value < 0.05 as statistically significant.

Discussion

Preclinical studies have indicated that MMP-11 mediates metastasis of cancer including MMP-11 expression regulates local or distant invasion in transgenic mice [25]. Furthermore, knockdown of MMP-11 expression in the mouse hepatocarcinoma cell line Hca-F inhibits its metastatic proliferation to lymph nodes [26]. In the breast cancer microenvironment also found MMP-11-positive mononuclear inflammatory cells infiltration and facilitated to form metastases [27]. The role of MMP-11 in the metastatic process, however, might be complex and even dual, probably depending on different spatiotemporal factors [28]. When we examined the influence of the *MMP-11* gene upon the metastatic phenotype of HCC, we discovered a significantly higher likelihood of lymph node metastasis among patients carrying the rs28382575 C/T+C/C genotype as compared with T/T carriers. These results suggest that knockdown MMP-11 might be a valuable therapeutic strategy for HCC lymph node metastasis.

Table 5. Association of *MMP-11* genotypic frequencies with HCC laboratory status.

Characteristic	α-Fetoprotein[a] (ng/mL)	AST (IU/L)	ALT (IU/L)	AST/ALT ratio
rs738791				
CC	922.1 ± 477.2	57.01 ± 9.19	55.88 ± 8.61	1.18 ± 0.03
TC+TT	1020.9 ± 372.4	54.80 ± 4.38	52.60 ± 4.33	1.23 ± 0.05
p value	0.870	0.823	0.733	0.382
p value[b]	0.865	0.809	0.713	0.401
rs2267029				
TT	1280.4 ± 442.0	60.38 ± 8.47	58.11 ± 7.78	1.21 ± 0.05
AT+AA	708.9 ± 386.9	50.61 ± 3.50	49.48 ± 4.09	1.20 ± 0.03
p value	0.395	0.286	0.326	0.936
p value[b]	0.390	0.298	0.333	0.938
rs738792				
TT	1041.2 ± 407.8	61.93 ± 9.11	59.64 ± 8.35	1.21 ± 0.05
GT+GG	911.7 ± 432.1	49.83 ± 3.27	48.64 ± 3.84	1.20 ± 0.03
p value	0.828	0.212	0.232	0.894
p value[b]	0.823	0.197	0.217	0.892
rs28382575				
AA	886.2 ± 282.2	56.03 ± 5.00	54.40 ± 4.75	1.20 ± 0.03
AG+GG	3077.3 ± 3006.4	51.17 ± 10.60	46.89 ± 10.36	1.21 ± 0.09
p value	0.473	0.680	0.513	0.944
p value[b]	0.134	0.837	0.738	0.965
rs131451				
AA	1546.8 ± 584.5	55.17 ± 7.59	56.17 ± 8.62	1.17 ± 0.04
AG+GG	660.2 ± 328.8	56.20 ± 6.19	52.95 ± 5.27	1.22 ± 0.04
p value	0.187	0.918	0.750	0.375
p value[b]	0.143	0.916	0.729	0.416

Mann-Whitney U test was used between two groups.

[a] Mean ± S.E.

[b] Adjusted age, sex, drink, HBsAg, and anti-HCV.

* *p* value < 0.05 as statistically significant

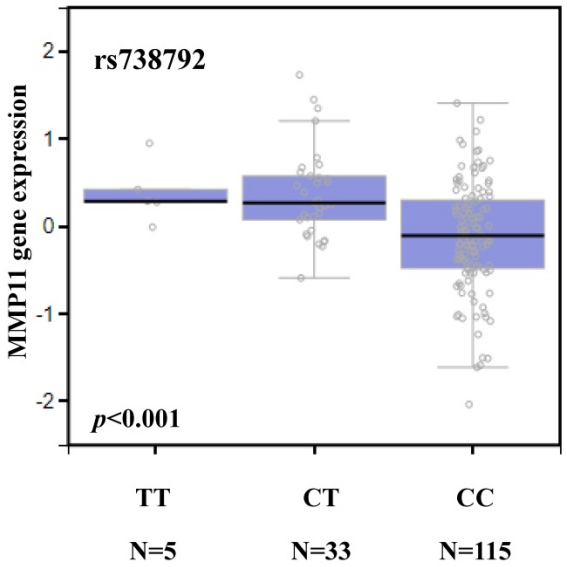

Figure 1. *MMP-11* displays a significant eQTL association with the rs738792 genotype in liver tissue (GTEx data set).

In view of the high occurrence rate and lethality of HCC, lowering the incidence and mortality rates present an important challenge. Infection with HBV or HCV, a history of liver cirrhosis, family history of HCC, and alcohol consumption are the dominant etiological factors for HCC in Taiwan [29]. In this study, no between-group differences were observed between the ratios of cigarette smokers/nonsmokers among controls (40.8:59.2, respectively) and HCC patients (41.6:58.4, respectively), whereas a higher proportion of HCC patients consumed alcohol (37.2%) compared with controls (14.5%). This suggests that alcohol consumption is a risk factor for HCC development. Chronic alcohol consumption promotes hepatobiliary tumors by increasing microRNA-122-controlled HIF-1α activity and stemness [30]. This is supported by findings from a swine model of chronic hypercholesterolemia, in which moderate alcohol consumption altered autophagy- and apoptosis-regulated pathways [31]. Interestingly, the findings indicated that a hypercholesterolemic diet supplemented with vodka appeared to induce pro-apoptotic pathways in liver tissue, whereas wine appeared to induce anti-apoptotic signaling. Our data is consistent with clinical evidence showing that alcohol consumption is a risk factor for HCC [32, 33]; we found that HCC patients who consumed alcohol were at higher risk of worsening disease.

Previous research has found no significant difference in the occurrence of oral squamous cell carcinoma (OSCC) amongst individuals with polymorphisms of the *MMP-11* gene in rs738791 [18]. In addition, the patients with rs738791 polymorphism and betel nut chewing increased risk for OSCC when compared to subjects with wild-type genes and without a history of betel nut chewing [34]. In this study, we found that the *MMP-11* rs738791 polymorphism was associated with HCC risk. These findings suggest that different *MMP-11* polymorphisms play different roles in cancer development. This study found that HCC patients with the *MMP1* rs738792 polymorphism had a higher risk of developing Child-Pugh B or C grade. Similarly, the *MMP-11* rs28382575 polymorphism was also associated with a higher risk of developing stage III/IV disease, large tumors and lymph node metastasis. It is established that MMP-11 expression controls the miR-125a regulated metastasis of HCC [16]. In addition, CD147 regulated HCC invasion and multidrug resistance through MMP-11 upregulation [35]. More research is required to determine whether an association exists among advanced-stage disease, *MMP-11* expression levels, and *MMP-11* genotype, and clarification is needed in regard to the effects of the *MMP-11* genotype on HCC risk.

In conclusion, the current study suggests a potentially clinically significant finding showing that several variants of the *MMP-11* gene are associated with the clinical status and susceptibility of HCC. However, we dose not recruited the survival results of HCC. Future research could evaluate the association

of *HMGB1* polymorphisms with survival of HCC. We found that individuals carrying the CT+TT allele of the *MMP-11* SNP rs738791 were at higher risk of HCC than wild-type (C/C) carriers. Genetic variations in the gene encoding *MMP-11* may be a significant predictor of early HCC occurrence and a reliable biomarker for disease progression.

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Competing Interests

The authors have declared that no competing interest exists.

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