

Original Research Article

Matrix metalloproteinases genotyping and risk of Hepatocellular Carcinoma in HCV infected patients

ABSTRACT

The annual number of new cases of hepatocellular carcinoma (HCC) worldwide is over 1 million. In developing countries, HCC is attributed to chronic hepatitis C virus (HCV) infection. Functional gene polymorphism of matrix metalloproteinases (MMP) promoters is proved to be correlated with different cancers. This study examined the association between MMP1 -1607, MMP9-1562, MMP14-6727 and MMP14-6767 gene polymorphisms and risk of HCC in HCV infected patients. 160 HCC patients (91 with and 69 without HCV) and 140 healthy control subjects were enrolled. Genomic DNA was analyzed using restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) for MMP1 and MMP9 but real time PCR for MMP14 genotyping. MMP1-2G allele carriers had higher susceptibility of developing HCC in HCV infected patients. MMP9-1562 T/T genotype had high risk of developing HCC in HCV and non HCV related patients when compared to healthy controls. A significant lower risk for HCC was shown in individuals with MMP14-6767 G/A. The distribution frequency of MMP14-6767 G and MMP14-6727 C allele and homozygote genotype was significantly higher in HCC patients. MMP14-6767 G and MMP14-6727 C alleles might contribute to the prediction of susceptibility and pathological development of HCC in HCV infected patients.

keywords: metalloproteinases, gene polymorphism, HCV, risk of HCC

INTRODUCTION

Hepatocellular carcinoma (HCC) is considered as one of the most prevalent tumors with high incidence and mortality rates. HCC is the 6th most common cancer worldwide and the 3rd leading cause of cancer-related death (1). More than 80% of HCCs develop in Asian and African countries. In Africa, especially in Egypt, hepatitis C virus (HCV) is the most leading cause of the increased HCC incidence (2). Egypt is an endemic area of HCV. Globally, 1 in 50 people are infected with HCV, whereas

approximately 1 in 7 of Egypt's 83 million people tested positive for antibodies against HCV. However, nearly 1 in 10 people carry its viral RNA and are therefore chronically infected (3).

Cirrhosis and HCC are the most serious complications of chronic HCV infection (4). Donato et al. noted that the risk of HCC increased 17-fold among HCV-infected patients compared with anti-HCV negative controls. The exact pathogenesis of hepatic injury is not well understood in HCC (5). It has been suggested that HCV is not cytopathic to hepatocytes and that the immune response, especially cytotoxic T cell responses against HCV-infected hepatocytes, is the main cause of liver cell injury, fibrosis, cirrhosis and eventually HCC (6).

Matrix metalloproteinases (MMPs) are, zinc dependent proteolytic enzymes, cleave extracellular matrix (ECM) (collagen, laminin and fibronectin) as well as non-matrix substrates (growth factors, cell surface receptors, etc). MMPs have important functions in pathologic conditions that are characterized by the excessive degradation of ECM, such as tumor metastasis, rheumatoid arthritis and periodontal disease (7).

MMPs play an important role in several steps of cancer development by regulating cancer-cell growth, differentiation, apoptosis, invasion, metastasis, angiogenesis and immune surveillance (8). Several polymorphisms in the promoters of a number of MMP genes, which are thought to affect the respective MMP production in an allele-specific manner, have been well characterized. There is increasing evidence indicating that these functional polymorphisms may contribute to inter-individual differences in susceptibility to a wide spectrum of cancers (9, 10).

MMP1, also called interstitial collagenase-1 and MMP3, also called stromelysin-1, are two important members in MMPs family. They are neighbors located on 11q22 with 11 exons and 10 introns and play important roles in cancer development and metastasis. Moreover, gene polymorphisms in the promoter regions of MMP genes, including MMP-1, MMP-3 and MMP-9 were found (11).

MMP-1 is one of the principal proteinases that possess interstitial classes of proteolytic activity against collagens, the most abundant ECM proteins in fibrotic livers (12). The promoter regions of MMP1 and MMP3 genes are polymorphic and have allele-specific effects on regulation of MMP gene transcription. The promoter region of MMP1 contains a guanine insertion/deletion polymorphism at position -1607 relative to the transcriptional start site, with one allele having a single guanine nucleotide (1G) and the other having two (2G) (13).

MMP-9, also called gelatiase B, is the most complex member of MMP family. It is produced by macrophages and has proteolytic activity against major components of the basement membrane like collagen type IV & V, gelatin, fibronectin and elastin. MMP9 is responsible for extravasation, migration and tissue remodeling during chronic inflammation. The expression of MMP9 is up-regulated in various human cancers. The C to T substitution at position 1562 in the promoter region of MMP9 gene has a higher transcriptional activity of the T-allelic promoter. This might be caused by DNA protein interaction abolishment by the C to T substitution at this polymorphism site (11).

MMP14, also known as membrane type 1 matrix metalloproteinase, helps modify and breakdown various components of the extracellular matrix forming a pericellular collagenase against ECM. In different cell types, MMP14 acts on their surrounding environment inducing tissue remodeling, angiogenesis, invasion and metastasis by catalyzing pericellular collagenolysis (14). Chen et al. found that both MMP14 -6767 G/A and 7096 C/C exhibited a significant lower risk to have HCC. They also discovered that the distribution frequency of 165 T: 221 T: 6727 C: 6767 G: 7096 T: 8153 G haplotype and diplotype was significantly higher in the HCC patients than the healthy control subjects (15).

Comparing genetic information of HCC patients with control subjects and with HCV infected patients is particularly valuable to mark a target gene for predicting susceptibility and pathological development of HCC. This study aimed at evaluation of the association between MMP-1-1607 1G/2G, MMP-9-1562 C/T, MMP-14-6767 G/A and MMP-14-6727 C/G gene polymorphisms and the susceptibility of HCC in HCV infected patients in Egyptian population.

SUBJECTS AND METHODS

Participants

The subjects of this study were divided into 3 groups, group I: 140 healthy persons as control group (mean age 64 ± 2.3 , 73 males and 67 females), group II: 91 patients with HCV related HCC (mean age 63.0 ± 5.5 , 51 males and 40 females) and group III: 69 non HCV related HCC (mean age 65 ± 6.9 , 38 males and 31 females). Patients of group II were all positive for serum HCV RNA. All the participants were recruited from Zagazig Univeristy Hospitals.

The patients were diagnosed with HCC according to the characteristic criteria of the national guidelines for HCC, such as liver injury diagnosed by histology or

cytology irrespective of a-fetoprotein titer where imaging data showed one of following three conditions: (1) one or more liver masses ≥ 2 cm in diameter via both computed tomography and magnetic resonance imaging; (2) one imaging data with early enhancement and a high level of AFP ≥ 400 ng/ml; (3) one imaging data with early arterial phase contrast enhancement plus early venous phase contrast washout regardless of AFP level (16). The study was approved by the ethical committee of Faculty of Medicine, Zagazig University and written informed consent was obtained from all participants.

For all participants serum alanine transaminase (ALT) and aspartate transaminase (AST) (Elitech), serum tumor marker alpha fetoprotein (AFP) by ELISA (Equipar, Saronno, Italy) were measured.

DNA extraction

Genomic DNA was isolated and purified from 200 μ l whole blood (EDTA) using the protocol provided by the manufacturer (Qiagen GmbH, Hilden, Germany). The purified DNA was safely stored at -20°C for later use.

Genotype analysis of MMP-1-1607 and MMP-9-1562 gene polymorphisms

MMP1-1607 1G/2G (rs1799750) and MMP9-1562 C/T (rs3918242) gene polymorphisms were analyzed using polymerase chain reaction followed by the restriction fragment length polymorphism (PCR-RFLP) method according to Okamoto et al (2010) (17). Polymerase chain reaction (PCR) was performed in a final volume of 50 μ l containing 10 μ l (100 ng) of genomic DNA, 1 μ l of each primer (0.5 μ M), 25 μ l of 2x Super Hot PCR Master Mix and 13 μ l deionized water. The amplification was carried out using thermal cycler 480, Perkin Elmer (Norwalk, CT 06856, USA). The PCR products were digested with 5 U of the restriction enzyme listed in Table 1 at 37°C for 3 h (Fast Digest, Thermoscientific), and the amplified products were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide using Submarine Gel Electrophoresis System (Pharmacia Biotech by SEMKO AB, Sweden) and submarine chamber (Maxicell, EC360, M-E-C apparatus Co. St Petersburg, Florida, USA). The gel was visualized under a UV transilluminator with Gene RulerTm 100 base pair DNA ladder (Fermentas Canada Inc, Burlington, Ontario, Canada). Primers sequence, PCR protocol, restriction enzymes and length of expected bands for MMP1-1607 1G/2G and MMP9-1562 C/T gene polymorphisms are shown in Table 1.

133 *Allele discrimination of MMP-14-6727 and MMP-14-6767 by Real-Time PCR*

134 The allelic discrimination of the MMP14-6727 (rs2236302) and MMP14-6767
 135 (rs1042704) gene polymorphisms were assessed using TaqMan assay (Applied
 136 Biosystems) with the Stratagene, MX3000P quantitative PCR System (Agilent
 137 technologies) and analyzed using MxPro QPCR Software (Agilent technologies).
 138 Table 2 lists the primers and probe sequences for each polymorphism. Primers were
 139 designed according to NCBI PRIMER-BLAST software (Washington, USA) from
 140 www.ncbi.nlm.nih.gov/tools/primer-blast. The final volume for each reaction was
 141 25µl, containing 12.5 µl 2X TaqMan Universal Master Mix (Applied Biosystems),
 142 0.5 µM TaqMan probes, 1 µM of each primer 4.5 µl deionized H₂O and 5 µl genomic
 143 DNA. The real time PCR reaction included pre-incubation at 50°C for 2 min and
 144 initial denaturation step at 95°C for 10 min, followed by 45 cycles, each consisting of
 145 95°C for 30 s and 60°C for 1 min.

146 **STATISTICAL ANALYSIS**

147 Statistical analysis was performed using SPSS version 11 (Chicago, IL, USA)
 148 statistical package. Continuous variables were expressed as means ± SD. Two-tailed t
 149 test was used for continuous variables, and Chi-square (χ^2) test for categorized
 150 variables. Hardy-Weinberg equilibrium (HWE) analysis were performed to compare
 151 observed and expected genotype frequencies using χ^2 test. In addition, odds ratios
 152 (ORs) and 95% confidence intervals (CIs) were calculated as a measure of the
 153 association of the MMP1, 9 and 14 polymorphisms with HCC development. A
 154 difference was considered significant if p was <0.05.

155 **RESULTS:**

156 *Baseline characteristics of the studied groups*

157 There were insignificant difference as regards both age and sex ($p>0.05$)
 158 between HCC patients and controls. In the HCC groups, There was a significant
 159 difference of the mean values of serum ALT, AST and AFP levels ($P>0.05$) in
 160 relation to control group (Table 3).

161 *Genotype and allele distributions of MMP-9 gene polymorphisms*

162 In our recruited groups, all analyzed gene markers were in Hardy–Weinberg
 163 equilibrium ($P>0.05$). Table 4 shows the genotypes distribution and allele frequencies
 164 in MMP1-1607 (1G/2G), MMP9-1562 (C/T), MMP14-6727 C/G and MMP14- 6767
 165 G/A in HCV and non-HCV related HCC patients as compared to healthy control. For

MMP1- 1607 (1G/2G), significant higher representation of the 2G/2G genotype and 2G allele frequency was found in the HCV related HCC group ($P= 0.03$ and $P= 0.01$ respectively) and not in non HCV related HCC when compared to healthy control group. HCC risk in 2G/2G genotype is 1.91-fold (95%CI, 1.06-3.42) and is 1.62-fold (95%CI, 1.11-2.35) in 2G carriers in HCV related HCC when compared to healthy individuals. There was no significant difference in representation of 1G genotype between different studied groups.

RFLP-PCR genotyping of 1562 C/T in the promoter of MMP-9 gene revealed a high significant increase in the TT genotype frequency and T allele frequency in patients with HCV related HCC as compared to that in normal individuals ($P<0.001$). In addition, there was a significant increase in TT genotype frequency and T allele frequency in overall HCC patients when compared to normal individuals ($P=0.001$ and $P<0.001$ respectively). However, there was insignificant difference in the genotype distribution between non HCV related HCC patients and normal individuals ($P>0.05$). A significant higher representation of the MMP9-1562 T/T genotype and T allele frequency was found between HCV related HCC group as compared to non HCV related HCC group ($P= 0.04$ and $P= 0.01$ respectively). The risk of HCC development in MMP9-TT genotype was 3.14-fold (95%CI, 1.76-5.61) in HCV infected patients as compared to healthy control and was 2.01-fold (95% CI, 1.03–3.90) as compared to non HCV related HCC (Table 4).

Real time allele discrimination results revealed significant high distribution of MMP14-6727 C and MMP14-6767 G alleles and homozygous genotypes in both HCV related HCC patients ($P<0.05$) and in overall HCC patients ($P<0.05$) when compared to healthy individuals. OR and its 95% CI of MMP14-6727 C/C and MMP14-6767 G/G, 2.7 (1.26-5.77) and 5.18 (1.50-17.90) respectively, showed a higher risk for development of HCC in HCV infected patients and overall HCC patients. However, MMP14-6727 G and MMP14-6767 A allele frequency exhibited a significant ($P= 0.008$ and $P= 0.004$ respectively) lower risk of 0.34-fold (95% CI, 0.20-0.79) and 0.20-fold (95% CI, 0.06-0.67), respectively, to have HCC compared with their corresponding wild-type allele in overall HCC and in HCV related HCC patients (Table 4).

DISCUSSION:

Various studies have suggested strong association between MMPs gene polymorphism and the risk of different cancers development (4, 6, and 17). Our study

is one of few researches that examined the association between MMP-1, 9 and 14 gene polymorphisms and risk of developing HCC in HCV infected patients in Egypt. Hepatitis virus infection is associated with the increase of oxidative stress in liver cells and results in DNA changes and instability, thus, increasing the risk of developing cirrhosis and/or HCC (18)

Our work reported that the frequency of MMP-1-1607 2G allele carriers and homozygotes genotype was significantly increased in HCC patients with HCV as compared to healthy controls. This agrees with what Okamoto et al (12) and Zhai et al (19) who studied Japanese and Chinese populations respectively.

However, Peng et al. reported that a significant association between MMP-1 1G/2G polymorphism and cancer risk in their meta-analysis which did not include hepatic cancer. They suggested that the MMP-1 -1607 1G/2G might play different roles in different types of cancers. One factor contributing to this discrepancy is that different human tissues might have different expression profiles of MMP-1 and transcription factors, and thus the same polymorphism might play different parts in different tissues. Another factor is that different cancers may be influenced by different environmental factors, which may support or decline the association with promoter polymorphisms of MMP-1 (20). It would be expected that the MMP genotypes would alter the risk of HCC under specific conditions such as exposure to cigarette smoke and alcoholic consumption. In fact, many factors such as chronic infection with HBV, male gender, family history, smoking, and alcoholic consumption have been shown as self-regulating risk factors for HCC (21, 22). HCV infection might be a contributing factor for the association of 2G allele with HCC development.

We also observed a significant relationship between MMP-9 -1562 T/T genotypes and T allele carriers with the development of HCC in overall cases and in HCV infected patients but there was no significant difference in the MMP-9 TT genotype and the T allele between HCC patients without HCV viral hepatitis when compared to healthy subjects. However, Okamoto et al., found a positive relationship between MMP-9 T carriers and poor HCC differentiation, but could not demonstrate a significant relationship with the development of HCC (17). Matsumura et al., found significant associations between this polymorphism and the invasive phenotype of gastric cancer (23).

233 Zhang et al. reported that MMP-9 expression levels could be influenced by
234 genetic variation in MMP-9 gene promoters. The sequences in the promoter region
235 containing the MMP9-1562 C/T polymorphic site constitute a chief regulatory
236 element which appears to be a binding site for a transcription repressor protein.
237 Therefore, C/T substitution at the polymorphic site eliminates the DNA-protein
238 interaction, resulting in a higher activity of the T-allelic promoter (24).

239 The prevalence of the MMP-9 C allele was found to be significantly greater in
240 liver cirrhosis patients than in chronic hepatitis patients, suggesting that the MMP-9
241 C/C genotype and C allele carriers may contribute to the progression of liver fibrosis.
242 Hence, MMP-9 CC genotype with low transcriptional activity has lower production of
243 MMP-9 in the fibrotic liver, resulting in rapid progression of liver fibrosis due to
244 decreased degradation of the ECM (12).

245 MMP-14, which initiates liver metastasis, is over-expressed in HCC (25). Our
246 results revealed that MMP-14 6767 A and 6727 G allele carriers have a lower risk for
247 HCC compared with their corresponding wild-type genotypes. The 6767 G/A
248 polymorphism cause asparagine amino acid in the protein residue to be substituted for
249 aspartic acid. This might alter the original function of MMP-14 protein. A allele
250 might reduce the bioactivity or expression of MMP-14, and the cellular behaviors are
251 thereby strange. This provides a logical explanation for the low risk to have HCC
252 (15).

253 MMP14-6727 C/G polymorphism might be able to change the translational
254 rate or mRNA stability of MMP-14 protein. It is a synonymous SNP that is located in
255 exon 5. However, its association with cancer has not been reported (26).

256 In conclusion, genotyping of related SNPs might provide a simple and
257 valuable method to predict the risk and the prognosis of cancer. MMP-1 -1607 2G
258 allele carriers would increase the risk of HCC only in HCV infected patients. People
259 with MMP-9-1562 T, 6727 C and 6767 G allele carriers and homozygote genotype
260 might contribute to the prediction of susceptibility and pathological development of
261 HCC.

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 334 CY, Chen F, Kulke MH, Heist RS, Christiani DC (2011): Association between
 335 Polymorphisms in Cancer-Related Genes and Early Onset of Esophageal
 336 **Table 1:** The primers sequence, PCR protocol, restriction enzymes, and length of
 337 products for genotyping of MMP1- 1607 (1G/2G), and MMP9-1562 (C/T) gene
 338 polymorphisms

339

	Primer sequence (5'-3')	PCR protocol	Length of PCR product	Restriction Enzyme	Length of digested products
MMP1-1607 (1G/2G) rs; 1799750	F: TCGTGAGAATGTCTTCCATT R: CTTGGATTGATTGAGATAAGTGAAATC	-1 cycle at 95 °C for 1 min. -35 cycles at 95 °C for 1 min, 55 °C for 30 sec and 72 °C for 30 sec. -1 cycle at 72 °C for 5 min.	234 bp	<i>XmnI</i>	2G allele: 117 bp 1G allele: 89&28 bp
MMP9-1562 (C/T) rs; 3918242	F: GCCTGGCACATAGTAGGCC- R: CTCCTAGCCAGCCGGCATC	-1 cycle at 95 °C for 2 min. -35 cycles at 95 °C for 45 sec, 67 °C for 45 sec and 72 °C for 45 sec. -1 cycle at 72 °C for 5 min.	870 bp	<i>SphI</i>	C allele: 435 bp T allele: 247&188 bp

340

341 **Table 2:** The primers sequence, probe sequence, PCR protocol and reference SNP of
 342 MMP14-6727 C/G and MMP14-6767 G/A gene polymorphisms

343

	Primer sequence (5'-3')	Probe sequence (5'-3')	PCR protocol
MMP14-6727 (C/G) rs; 2236302	F: GAGCATTCCAGTGACCCCTC R: GGGCAGCACAAAATTCTCCG	Probe-G: VIC- GTAAAACGGTGCCA Probe-C: FAM- GTAAAAGGGTGCCA	Pre-incubation at 50°C for 2 min and initial denaturation step at 95°C for 10 min, followed by 45 cycles, each consisting of 95°C for 30 s and 60°C for 1 min.
MMP14-6767 (G/A) rs; 1042704	F: GGAGAATTTGTGCTGCCCG R: GGGTGTAGACTACTCGCCATA	Probe-A:VIC- CATCATTGGGCAGCA Probe-G:FAM- CATCATCGGGCAGCA	

344

345 **Table 3:** Demographic characteristics and clinicopathological parameters of the
 346 studied groups

Parameters	HCV related HCC (n = 91)	p	Non HCV related HCC (n = 69)	p	Control group (n = 140)
Age (years)	63±5.5	0.06	65±6.9	0.12	64±2.3

Sex (male/female)	51 (56%) / 40(44%)	0.56 [*]	38 (55%) / 31 (45%)	0.69 [*]	73 (52.1%) / 67 (47.9%)
Alanine Transaminase (IU/L)	62±5	< 0.001	61±1.1	< 0.001	22±6
Aspartate Transaminase (IU/L)	85±9	< 0.001	71±8	< 0.001	24±9
AFP (ng/mL)	486 ± 394 <400 (12%) ≥400 (88%)	< 0.001	463± 287 <400 (15%) ≥400 (85%)	< 0.001	11±2.2
Tumor size (cm)	3.6±2.3	-----	2.4±1.1	-----	0

347 ^{*}p calculated by Chi square (χ^2) test versus control

348

349 **Table 4: Table 4: Genotype and allele frequencies of MMP polymorphisms in**
350 **patients with hepatocellular carcinoma and controls and OR for hepatocellular**
351 **carcinoma risk**

Polymorphism	Control n, (%)	HCV related HCC n, (%)	OR (95% CI)	P	Non HCV related HCC n, (%)	OR (95% CI)	P	Overall individual cases n, (%)	OR (95% CI)	P
MMP1 –										
1607	46 (32.9)	20(21.9) 39(42.9)	0.58(0.31- 1.06)	0.07 0.75	22 (31.9)	0.96(0.5 2-1.77)	0.8 9	42(26.2) 69(43.1)	0.73(0.4 1.1)	0.21 0.74
1G/1G	63 (45.0)	32(35.2)	0.92(0.84- 1.56)	0.03	30 (43.5)	0.94(0.5 3-1.68)	0.8 4	49(30.7) 153(47.8)	4- 0.93(0.5 1.1)	0.10 -----
2G/2G	31(22.1)	79 (43.4)	1.91(1.06- 3.42)	----- 0.01	17 (24.6)	1.15(0.5 8-2.26)	0.6 9	167(52.2)	1.55(0.9 2-2.62)	----- 0.07
1G allele frequency	155 (55.4)	103(56.6) ^a	Reference 1.62(1.11- 2.35)		74 (54.0)	Referen ce 1.07(0.7 1-1.61)	----- 0.7 4		Referen ce 1.35(0.9 8-1.87)	
2G allele frequency	125 (44.6)				64 (46.0)					
MMP9 –										
1562	51 (36.4)	12 (13.2)	0.27(0.13- 0.53)	<0.001 0.86	17 (24.6)	0.57(0.3 0-1.10)	0.0 9	29 (18.1) 70(43.8)	0.39(0.2 3-0.66)	0.000 3
C/C	60 (42.9)	38 (41.7)	0.96(0.56- 1.63)	<0.001	32 (46.4)	1.15(0.6 6-2.06)	0.6 3	61 (38.1) 128(40.0)	1.04(0.6 2.36(1.4 0-3.96)	0.89 ----- <0.00
C/T	29 (20.7)	41 (45.1) ^b	3.14(1.76- 5.61)	----- -- <0.001	20(29.0)	1.56(0.8 1-3.03)	0.1 8			
T/T					66	Referen	-----	192(60.0)	Referen	1

frequency	9) 118(42.1)	62(34.1) 120 (69.9) ^c	2.66(1.80-3.92)		(47.8) 72 (52.2)	ce 1.45(0.9 9-2.26)	- 0.0 5		ce 2.06(1.4 9-2.85)	
MMP14										
+6727	105(75.0)	81(89.0) 9(10.0)	2.7(1.26-5.77)	0.009	57(82.7)	1.58(0.7 6-3.29)	0.2 1	138(86.2)	2.09(1.1 6-3.77)	0.013
C/C				0.02	)			)		0.03
C/G		1(1.0)	0.38(0.17-0.86)	0.65	11(15.9)	0.67(0.3 1-1.42)	0.2 9		0.50(0.2 7-0.93)	0.42
G/G	31(22.1)	171(94.0)	0.38(0.04-3.43)	-----	1(1.4)	0.50(0.0 5-4.56)	0.6 7	20(12.5) 2(1.3)	0.43(0.0 8-2.39)	-----
C allele		)		0.008						0.01
frequency	4(2.9)	11(6.0)			125(90.6)	Referen	-----	296(92.5)	Referen	
G allele			Reference			ce	-	)	ce	
frequency	241(86.1)		0.34(0.20-0.79)		13(9.4)	0.64(0.3 3-1.23)	0.1 9	24(7.5)	0.50(0.2 9-0.86)	
	39(13.9)									
	)									
MMP14										
+6767	119(85.0)	88(96.7) 3(3.3)	5.18(1.50-17.90)	0.004	62(89.9)	1.56(0.6 3-3.88)	0.3 3	150(93.8)	2.65(1.2 0-5.84)	0.01
G/G				0.006	)			)		0.011
G/A		0(0)	0.20(0.06-0.71)	-----	6(8.7)	0.57(0.2 2-1.49)	0.2 3	9(5.6) 1(0.6)	0.36(0.1 6-0.81)	1
A/A	20(14.3)	179(98.4)	-----	-----	1(1.4)	2.04(0.1 2-33.18)	1		0.87(0.0 5-14.11)	-----
G allele		)	---	0.004				309(96.6)		0.02
frequency	1(0.7)	3(1.6)			130(94.2)	Referen	-----	)	Referen	
A allele		^d	Reference			ce	-	11(3.4)	ce	
frequency	258(92.1)		0.20(0.06-0.67)		8(5.8)	0.72(0.3 1-1.67)	0.4 4		0.42(0.2 0-0.88)	
	22(7.9)									

352 ^ap= 0.07; OR=1.51; 95% CI (0.97–2.35); versus non HCV–related HCC

^bp= 0.04; OR=2.01; 95% CI

353 (1.03–3.90); versus non HCV –related HCC

354 ^cp= 0.01; OR=1.77; 95% CI (1.13–2.79); versus non HCV –related HCC

^dp= 0.06; OR=0.27; 95% CI

355 (0.07–1.05); versus non HCV –related HCC