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Study of matrix metalloproteinases-3 and -9 genepolymorphisms in the prognosis of hepatocellular carcinoma in Egyptian population

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ABSTRACT

This study aimed to study the relationship between the gene polymorphisms of MMP-3 and MMP-9 and the prognosis of hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) in Egyptian population. Methods: The study enrolled 180 Egyptian individuals classified into 3 groups Group I: consists of 60 apparently healthy individuals served as control Group II: consists of 60 individuals diagnosed with HCV Group III: consists of 60 individuals diagnosed with HCC. Gene polymorphisms of MMP-3 -1, 171 5A/6A, and MMP-9 -1, 562 C/Tusing restriction fragment length polymorphism (RFLP) for amplified genomic DNA were analyzed. Results: In HCC prognosis, MMP-3 5A carriers hadhigher probability of developing HCC when compared to healthy individuals. Conclusion: MMP-3 5A allele may bea cooperative risk factor for poor prognosis in HCC patients, suggesting that further studies with larger sample size should be investigated to ensure that this gene polymorphism might be a potential marker for predicting the prognosis of HCC patients in Egyptian patients.

KEYWORDS

MMP-3; MMP-9;
Gene polymorphism;
Hepatocellular carcinoma;
Prognosis;
Hepatitis virus C.

INTRODUCTION

HCC is the most common primary liver cancer. The annual number of new cases of HCC worldwide is over one million, making it the 5th most common cancer worldwide and the 3rd leading cause of cancer-related death. Men are more likely to be affected than women with HCC. This trend was observed in almost all countries[1]. In developing countries, the major concern in HCC is chronic HCV infection. In Egypt, the prevalence of HCV infection among general population has been estimated to be around 14%. Chronic HCV infection mostly leads to hepatic cirrhosis before developing HCC[2,3].

MMPs are multifunctional proteins and that their roles in cancer are much more complex than originally thought. In addition to ECM degradation, there is now considerable evidence for their involvement in regula-

tion of cell growth, survival, differentiation, inflammation and angiogenesis through precise cleavage of various molecules[4]. The 23 members of this family of endopeptidases share a catalytic domain, a pro-peptide and a hemopexin-like C-terminal domain[5]. Functional polymorphisms in these genes, such as single nucleotide polymorphisms (SNPs) located in promoter regions, may influence the expression or activity of the proteins and thus modify cancer susceptibility and prognosis[6]. Matrix degradation in the liver is due to the action of at least four of these enzymes: MMP-1, MMP-2, MMP-3, and MMP-9[7].

MMP-3 is a member of the stromelysin subfamily of MMPs, which comprises stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), and stromelysin-3 (MMP-11). Stromelysin-1 is overexpressed in a wide variety of tumour types, where it is almost exclusively found in the tumour stroma, i.e. fibroblasts, endothelial cells, immune cells[4]. MMP-3 has a broad spectrum of ECM substrates including fibronectin, laminin, vitronectin, different collagens, as well as other MMPs such as pro-MMP-2 or -9, leading to local invasion and metastasis by facilitating degradation of ECM and promoting infiltration of tumor cells through the basal membrane[8,9]. MMP-3 is thought to play a significant role in ECM degradation in chronically diseased livers, in which fibrosis is developing. MMP expression during the course of chronic hepatitis C has been studied by Lichtinghagen et al.[10]. They found that hepatic MMP-3 expression was unrelated to disease stage, but was determined by the MMP-3(-1171) promoter polymorphism[7] he gene coding for MMP-3 is located on the long arm of chromosome 11 in regions 11q22.2– 22.3[11]. The polymorphism of the MMP-3 promoter region is the insertion/deletion of an adenosine (A) at position -1, 171 and has two alleles, one having a run of six adenosines (6A) and the other five (5A)[12]. Ye et al.[13] reported that the MMP3 -1171 5A allele has approximately 2-fold higher promoter activity than the 6A allele in in vitro experiments.

MMP-9 is a gelatinase type IV collagenase [gelatinase B] which plays an important role in tumor invasion and angiogenesis. MMP-9 expression has been found in a large variety of cell types, including epithelial cells, fibroblasts and endothelial cells. Expressions of MMP-9 in tumor tissues were of prognostic significance for poor overall survival of patients with gastric carcinoma and HCC[4,14]. MMP-9 is involved in the degradation of a broad spectrum of substrates, including collagen types IV, V, VII and X as well as gelatin, and also degrades the proteoglycans and the elastins[15]. Expression levels of MMP9 and protein function could be modified by functional SNPs in or around the MMP9 gene region, thus promoting tumor growth, invasion or metastasis[6]. MMP9 gene is located on chromosome 20q11.2– q13.1with a promoter gene polymorphism of a cytosine (C) to thymidine (T) substitution at position -1562. For MMP9 -1562 C>T polymorphism T allele had a higher promoter activity than the C allele, which appeared to be due to preferential binding of a putative transcription repressor protein to the C allelic promoter[16,17].

This study examined the association of MMP-3 and MMP-9 gene polymorphisms with the prognosis of hepatitis virus C (HCV)-related HCC patients in Egyptian Poulation.

SUBJECTS & METHODS

Site of the study

The study was carried out in Biochemistry and Internal Medicine Departments - Faculty of medicine, Zagazig university.

Subjects

The present study was carried out on 180 Egyptian individuals (71 females and 109 males) with age ranged from (39 to 61 years). They were divided into the following groups: Group I: consists of 60 apparently healthy individuals served as control (39 males and 21 females) with ages ranged from 39 to 61 with a mean value ± SD of (50.32 ± 6.84) Group II: consists of 60 individuals diagnosed with HCV (36 males and 24 females with ages ranged from 43 to 61 with a mean value ± SD of 50.5 ± 5.45)Group III: consists of 60 individuals diagnosed withHCC (34 males and 26 females) with ages ranged from 44 to 59 with a mean value ± SD of (52 ± 4.48)

Inclusion criteria

All participants (except control) should be positive for serum HCV RNA.

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Exclusion criteria

Patients who had chronic hepatitis B virus infection, alcoholism, primary biliary cirrhosis, or autoimmune liver disease will be excluded.

All individuals were subjected to the following:-

1 Measurement of AFP level by the third generation ELISA using kits from the Equipar (Saronno, Italy).
2 Measurement of liver enzymes (ALT and AST) by Bayer Opera Chemistry System (Diagnostic Division Tarrytown, NY USA).
3 Determination of MMP-3, 9 gene polymorphism by PCR amplification followed by restriction Fragment length polymorphism (RFLP) and gel electrophoresis.

DNA extraction

Genomic DNA was isolated from 3 ml venous blood sample withdrawn on EDTA using genomic DNA purification kit (Fermentas) according to the manufacturer's instructions.

Genotype analysis

Gene polymorphisms were detected through PCR amplification followed by digestion using restriction endonuclease enzymes for RFLP analysis. MMP3 gene 5A/6A polymorphism was genotyped using the forward primer (5'-GGTTCTCCATTCCTTTGATGGGGGGAAAgA-3'); and the reverse primer (5'-CTTCCTGGAATTCACATCACTGCCACCACT-3'), according to previous reports[18]. The second nucleotide A in the forward primer 3' end was substituted by G in order to facilitate change in the 5A allele and generate the recognition sites for Psy I (Tth111 I) (GACN/NNGTC). The genotyping of MMP-9 promoter at position -1562 was detected by using the forwardprimer, (5'-GCCTGGCACATAGTAGGCCC-3') and the reverse primer, (5'-CTTCCTAGCCAGCCGGCATC-3') as previously described[19].

PCR reaction for both polymorphisms

PCR reaction was performed in a final volume of 50µl that contained : 2X PCR Mix: 25 µl, Primer mix (2.5 µM or 1/40 0f dilution 100 µM stock): 1µl for each primer, Genomic DNA: 5 µl and Deionized water: 18 µl. The amplification was carried out using DNA thermal cycler 480, PERKIN ELMER (Norwalk, CT 06856, USA), Serial No. P 16462.

PCR conditions for MMP 3 polymorphism were; 5 min cycle for initial denaturation at 95 ÚC; 30 cycles at 94 ÚC for 30 sec for denaturation, 67 ÚC for 30 sec for annealing and 72 ÚC for 45 sec for extension, followed by 1 cycle at 72 ÚC for 5 minutes for final extension[18].

PCR conditions for MMP 3 polymorphism were; 2 min cycle for initial denaturation at 95 ÚC; 30 cycles at 95 ÚC for 45 sec for denaturation, 67 ÚC for 45 sec for annealing and 72 ÚC for 45 sec for extension, followed by 1 cycle at 72 ÚC for 5 minutes for final extension[19].

Restriction enzyme digestion

The PCR products were digested with restriction endonucleases (Fast Digest, Thermo Scientific) and subjected to electrophoresis on a 2% agarose gel and the bands were visualized by ethidium bromide staining under U/V light.

For MMP-3 -1171 (5A/6A)

Digestion of the PCR fragments with Tth111 I produced 130, 97 and 33 bp for 5A/6A allele, 97 and 33 bp for 5A allele and 130 bp for 6A allele[18].(Figure 1).

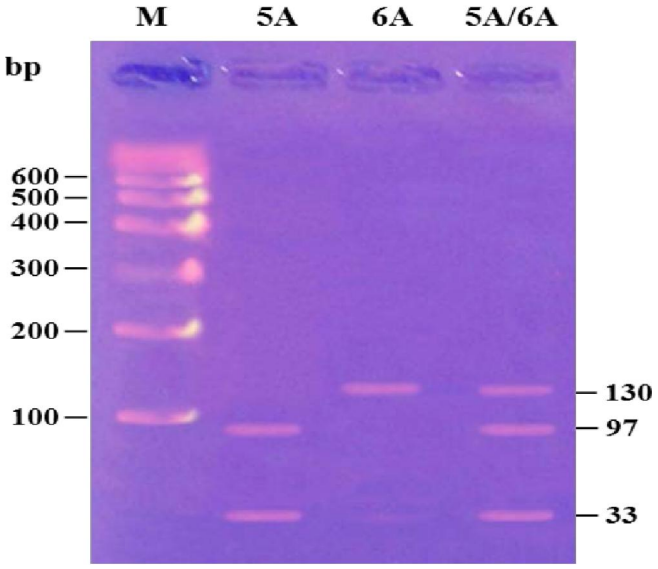

Figure 1 : Shows a 2% agarose gel picture, stained with ethidium bromide, products digested with Tth111 I .M lane: 100 bp - 1kb DNA ladder (Fermentas); lan 1: 5A homozygote (97 bp + 33 bp); lan 2: 6A homozygote (130 bp); lan 3: 5A/6A heterozygote (130 bp + 97 bp + 33 bp).

For MMP-9 -1562 (C/T)

Digestion of the PCR fragments with Sph I restriction endonuclease produced 435, 247 and 188 bp for C/T allele, 188 and 247 bp for TT allele and 435 bp for CC allele (not cleaved by SphI)[19].(Figure 2).

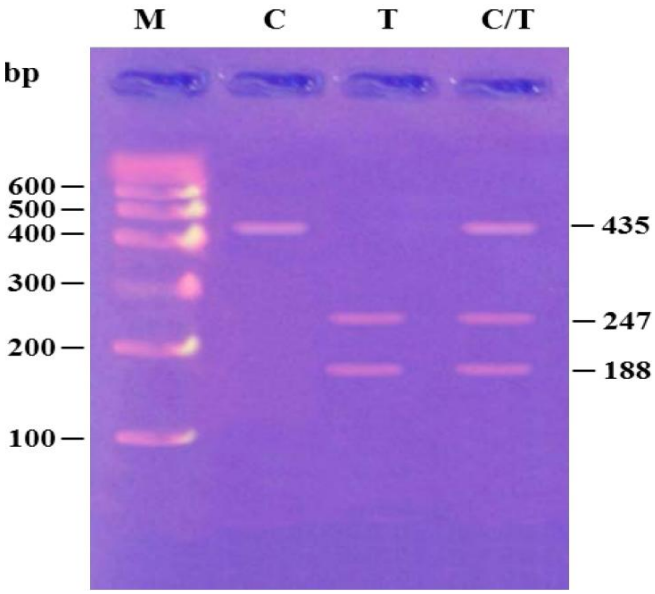

Figure 2 : Shows a 2% agarose gel picture, stained with ethidium bromide, products digested with Sph I. M lane: 100 bp - 1kb DNA ladder (Fermentas) ; lan 1: C homozygote (247 bp + 188 bp); lan 2: T homozygote (435 bp); lan 3: C/T heterozygote (435 bp + 247 bp + 188 bp).

Statistical methods

All statistical analysis was performed using the statistical package for social science (SPSS) version 11 (Chicago, IL, USA)[20]. Data were statistically described in terms of mean±standard deviation (±SD), range, or frequencies (number of cases) and percentages when appropriate. Odds ratio (OR) and 95% confidence interval (95%CI) were calculated for all studied polymorphism haplotypes and alleles between cases and controls. P values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of the study participants

The study was carried out on 180 individuls classified into 3 groups: Group I: Control group (n=60): Including 39 (60.6%) males and 21 (39.4%) females with ages ranged from 39 to 61 with a mean value ± SD of 50.32 ± 6.84). Group II: HCV group (n=60): Patients positive for serum HCV Including 36 (60%) males and 24 (40%) females with ages ranged from 43 to 61 with a mean value ± SD of 50.5 ± 5.45). Group III: HCC group (n=60): patients diagnosed with HCC including 34 (56.7%) males and 26 (43.3%) females with ages ranged from 44 to 59 with a mean value ± SD of 52 ± 4.48). TABLE 1 shows the laboratory data of the individuals in the three groups.

Genotype distributions & allelefrequencies of MMP-3and -9 gene polymorphisms.

Genotype distributions for MMP-3.

5A/5A genotype: a higher representation of the 5A/5A genotype was found in HCV group as compared to control group & in HCC group as compared to control group. Although statistical significance was observed between Control & HCV (P=0.04; OR = 5.118; CI = 1.057-24.789), this significance disappeared when comparing Control to HCC (P=0.103; OR = 3.83; CI = 0.762-19.258). 5A/5A genotype was underrepresented in the HCC group when compared to the HCV group (TABLE 2).

5A/6A genotype: 5A/6A genotype was underrepresented when comparing control & HCV group and control & HCC. A Slightly higher representation of the 5A/6A genotype was found between HCV group and HCC group but didn't reach statistical significance (TABLE 2).

6A/6A genotype: There was no significant differ-

TABLE 1 : The association between serum AFP, ALT and AST level in different studied groups

Parameters	Controls (n =60)		HCV (n=60)		HCC (n=60)		
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	* P-value
AFP (ng/ml)	1.914	0.828	10.964	2.277	485.978	636.950	<0.001
ALT (U/L)	24.816	3.702	40.666	30.836	238.601	256.150	<0.001
AST (U/L)	26.683	4.416	46.883	41.884	203.249	220.283	<0.001

*** p>0.05 is considered non-significant; p<0.05 is considered significant.**

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TABLE 2 : Genotypes distributions of MMP-3, 9 gene polymorphisms among control and different patient groups.

Polymorphism	Cases n= (%)	Control n=60 (%)	OR	95 % CI	*P-value
MMP-3 gene polymorphism					
		HCV (n=60)			
6A	36 (60)	37 (61.7)		**1 (reference)**	
5A/6A	15 (25)	21 (35)	0.619	0.281 – 1.363	0.234
5A	9 (15)	2 (3.3)	5.118	1.057-24.789	0.04
		HCC (n=60)			
6A	37 (61.7)	37 (61.7)		1 (reference)	
5A/6A	16 (26.7)	21 (35)	0.675	1.474 -0.3095	0.324
5A	7 (11.6)	2 (3.3)	3.83	0.762 – 19.258	0.103
		HCV (n=60)			
C	40 (66.7)	35 (58.3)		**1 (reference)**	
C/T	17 (28.3)	20 (33.3)	0.7907	0.3636 - 1.7194	0.5535
T	3 (5)	5 (8.3)	0.5789	0.1320 - 2.5396	0.4688
		HCC (n=60)			
C	38 (63.3)	35 (58.3)		**1 (reference)**	
C/T	19 (31.7)	20 (33.3)	0.9268	0.4316 - 1.9902	0.8455
T	3 (5)	5 (8.3)	0.5789	0.1320 - 2.5396	0.4688

*** p>0.05 is considered non-significant; p<0.05 is considered significant.**

ence in representation of 6A genotype between different studied groups (TABLE 2).

There was no significant association in the characteristics of subjects among the three groups tested for the -1171 5A/6A polymorphism (P value >0.05)

Genotype distributions for MMP-9

T/T genotype: T/T genotype was underrepresented in HCV group as compared to control group & in HCC group as compared to control group. (TABLE 2).

C/T genotype: C/T genotype was underrepresented when comparing HCV & control groups. A Slightly higher representation of the C/T genotype was found between HCC group and HCV group but didn't reach statistical significance (TABLE 2).

C/C genotype: a higher representation of the C/C genotype was found in HCV group as compared to control group & in HCC group as compared to control group. C/C genotype was underrepresented in the HCC group when compared to the HCV group (TABLE 2).

There was no significant association in the characteristics of subjects among the three groups tested for the -1562 C/T polymorphism (P value >0.05).

Allele frequencies for MMP-3

5A allele: a higher representation of 5A allele was present in the HCV group when compared with both HCC group and Control but didn't reach statistical significance (TABLE 3).

6A allele: a higher representation of 6A allele was present in the control group when compared with both HCV group and HCC group(TABLE 3).

Risk Assessment: The association of 5A genotype and HCC showed that 5A/5A individuals have a 3.83 time higher probability of developing HCC when compared to healthy individuals. There was not any statistically significant allelic association between HCC group & Control group (P=0.229 and 0.443; OR=1.44 and 1.267 respectively) claiming only 5A/5A individuals are in risk of developing HCC; individuals without 5A allele may be protected from the disease.

Allele frequencies for MMP-3

C allele: a higher representation of C allele was present in the HCV group when compared with both HCC group and Control but didn't reach statistical significance(TABLE 3).

T allele: T allele was underrepresented in HCV group as compared to control group & in HCC group as compared to control group (TABLE 3).

Risk Assessment: No significant difference in C/T SNP of MMP 9 gene between HCV patients, HCC

TABLE 3 : Allele frequency of MMP-3, 9 gene polymorphisms among control and different patient groups

Polymorphism	Cases n= (%)	Control n=60 (%)	OR	*P-value
MMP-3 Allele frequencies				
HCV (n=60)				
6A	87 (72.5)	95 (79.17)	0.694	0.229
5A	33 (27.5)	25 (20.83)	1.44	
HCC (n=60)				
6A	90 (75)	95 (79.17)	0.789	0.443
5A	30 (25)	25 (20.83)	1.267	
MMP-9 Allele frequencies				
HCV (n=60)				
C	97 (71.67)	90 (86.67)	1.405	0.277
T	23 (28.33)	30 (13.33)	0.7113	
HCC (n=60)				
C	95 (80.83)	90 (86.67)	1.266	0.443
T	25 (19.17)	30 (13.33)	0.789	

*** p>0.05 is considered non-significant; p<0.05 is considered significant.**

patients and control group (TABLE 3)

DISCUSSION

This study evaluated the effect of -1171 (5A/6A) and -1562 (C/T) polymorphisms in thepromoter regions of human gelatinaseMMP9, and the human stromelysin MMP3 on the Prognosis of Hepatocellular Carcinoma in Egyptian population.

Ye et al.[13] reported that the MMP3 -1171 5A allele has approximately 2-fold higher promoter activity than the 6A allele in in vitro experiments. The results showed that 5A/5A individuals had 3.83 times higher probability of developing HCC when compared to healthy individuals but there was no statistically significant difference in 5A/6A SNP of MMP3 gene between HCC patients and control group. The study findings are in accordance with Okamoto K et al[12] who reported that MMP-3 5A allele is cooperative risk factors for poor prognosis in HCC Japanese patients in accordance with large CC diameter and decreased survival for 5A allele carriers (95, 92 cases and 83, 170 control respectively). In other carcinomas, Bradbury et al[21] reported that MMP3 -1171 polymorphism and its haplotypes are associated with increasedesophageal adenocarcinoma risk (In a Caucasian population, 313 cases & 455 controls).

There are also other studies that are not in agreement with this study findings, Zhai Y et al[22] who reported that there is no association between MMP3 -1171 polymorphism andHCC progression in Chinese patients(434 cases and 480 control). Also, Hyun Phil Shin et al[7] reported that MMP-3 gene polymorphisms could not account for the progression of HBV related liver cirrhosis, but carrying 5A carriers was associated with a low platelet count, which was important marker of liver cirrhosis although not diagnostic for liver cirrhosis. In other carcinomas, Früh M et al[23] who reported that there is no association between MMP3 -1171 polymorphism and esophageal adenocarcinoma risk (In a Caucasian population, 101 cases and 101 controls).

For MMP9 -1562 C>T polymorphism T allele had a higher promoter activity than the C allele, which appeared to be due to preferential binding of a putative transcription repressor protein to the C allelic promoter[16,17]. There was No significant relationship between MMP-9 -1562 C/T genotypes with either development or prognosis of HCC. Study findings are in accordance with Zhai Y et al[22] who reported that there is no association between MMP-9 -1562 C/T polymorphism and HCC progression in Chinese patients (434 cases and 480 controls). Although Okamoto K et al[13] reported a positive relationship between MMP-9 T carriers and poorer HCC differentiation; he could not detect a significant relationship between MMP-9 -1, 562 C/T genotypeswith either development or prog-

nosis of HCC. In other carcinomas, Xia et al[24] who reported that there is no association between MMP-9 -1562 C/T polymorphism and Esophageal Squamous Cell Carcinoma risk (In a Chinese population, 132 cases and 132 controls).

There are number of factors which affectunexpected results on polymorphism- disease association studies which also will explain why study results are not in agreement with previous mentioned studies. Ethnicities of the races; People of different populations have different genetic backgrounds and may be exposed to different environment factors, so the same polymorphism may play different roles in different populations. Control type also contributed to heterogeneity; the results from healthy controls are more reliable than those from diseased controls, as the frequency of MMP alleles in a diseased population may deviate from normal. In addition toSample size and Gender type were also a causal reason for heterogeneity. All these factors must be paid more interest in future studies.

REFERENCE

[1] SeneWaly Raphael, Zhang Yangde, Chen Yu Xiang; Hepatocellular Carcinoma: Focus on Different Aspects of Management, ISRN Oncology, 421673 **(2012)**.

[2] A.R.El-Zayadi, H.M.Badran, E.M.F.Barakat, M.E.D.Attia, S.Shawky, M.K.Mohamed, O.Selim, A.Saeid; Hepatocellular carcinoma in Egypt: A single center study over a decade, World J.Gastroenterol, **33**, 5193-5198 **(2005)**.

[3] N.M.Abdel-Hamid; Recent insights on risk factors of hepatocellular carcinoma, World J.Hepatol., **1**, 3-7 **(2009)**.

[4] Julie Decock, Sally Thirkettle, Laura Wagstaff, R.Dylan Edwards; Matrix metalloproteinases: protective roles in cancer, J.Cell.Mol.Med., **15**, 1254-1265 **(2011)**.

[5] M.J.Alexandra Langers, Hein.W.Verspaget, Daniel.W.Hommes, F.M.Cornelis Sier; Single-nucleotide polymorphisms of matrix metalloproteinases and their inhibitors in gastrointestinal cancer, World J.Gastrointest.Oncol., **6**, 79-98 **(2011)**.

[6] Guangfu Jin, Ruifen Miao, Zhibin Hu, Lin Xu, Xinen Huang, Yijiang Chen, TianTian, Qingyi Wei, Paolo Boffetta, HongbingShen; Putative functional polymorphisms of MMP9 predict survival of NSCLC in a Chinese population.Int.J.Cancer, **124**, 2172–2178 **(2009)**.

[7] Hyun Phil Shin, Joung Il Lee, Joo-Ho Jung, Sung-Vin Yim, Hyun Jeong Kim, Jae Myung Cha, Jong Beom Park, Kwang Ro Joo, Jae Seok Hwang, Byoung-Kuk Jang; Matrix Metalloproteinase (MMP)-3 Polymorphism in Patients with HBV Related Chronic Liver Disease. Dig Dis Sci., **53**, 823–829 **(2008)**.

[8] H.Husslein, S.Haider, G.Meinhardt, J.Prast, S.Sonderegger, M.Knöfler; Expression, Regulation and Functional Characterization of Matrix Metalloproteinase-3 of Human Trophoblast, Placenta, **3**, 284–291 **(2009)**.

[9] Shumei Fang, Xia Jin, Rui Wang, Yan Li, Wei Guo, Na Wang, Yimin Wang, Denggui Wen, Lizhen Wei, Jianhui Zhang; Polymorphisms in the MMP1 and MMP3 promoter and non-small cell lung carcinoma in North China. Carcinogenesis, **26**, 481-486 **(2005)**.

[10] R.Lichtinghagen, M.J.Bahr, M.Wehmeier, D.Michels, C.I.Haberkorn, B.Arndt, P.Flemming, M.P.Manns, K.H.Boeker; Expression and coordinated regulation of matrix metalloproteinases in chronic hepatitis C and hepatitis C virus-induced liver cirrhosis. Clin Sci., **105**, 373–382 **(2003)**.

[11] K.K.Shalia, V.K.Shah, M.R.Mashru, S.L.Soneji, J.B.Vasvani, S.Payannavar, A.Walvalkar, R.Mokal, S.S.Mithbawkar, M.Bootwalla, P.Sadvekar, P.K.Thakur; Matrix Metalloproteinase-3 (MMP-3)-1612 5A/6A Promoter Polymorphism In Coronary Artery Disease In Indian Population. Indian Journal of Clinical Biochemistry, **2**, 133-140 **(2010)**.

[12] K.Okamoto, C.Ishida, Y.Ikebuchi, M.Mandai, K.Mimura, Y.Murawaki, I.Yuasa; The genotypes of IL-1 beta and MMP-3 are associated with the prognosis of HCV-related hepatocellular carcinoma, Intern Med., **49**, 887-895 **(2010)**.

[13] S.Ye; Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases, Matrix Biol., **19**, 623–629 **(2000)**.

[14] DenizNart, BanuYaman, FundaYilmaz, Murat Zeytunlu, ZekiKarasu, Murat Kilic; Expression of Matrix Metalloproteinase-9 In Predicting Prognosis of Hepatocellular Carcinoma After Liver Transplantation, Liver Transplantation, **16**, 621-630 **(2010)**.

[15] D.E.Kim, J.Y.Kim, D.E.J.Schellingerhout; Protease imaging of human atheromata captures molecular

information of atherosclerosis, complementing anatomic imaging, ArteriosclerThromb.Vasc.Bio., **30**, 449–56 **(2010)**.

[16] Bo Peng, Lihuan Cao, Xiaopin Ma, Wenzhang Wang, Dan Wang, Long Yu; Meta-analysis of association between matrix metalloproteinases 2, 7 and 9 promoter polymorphisms and cancer risk, Mutagenesis, **25**, 371–379 **(2010)**.

[17] Kinya Okamoto, Kenichi Mimura, Yoshikazu Murawaki, Isao Yuasa; Association of functional gene polymorphisms of matrix metalloproteinase (MMP)-1, MMP-3 and MMP-9 with the progression of chronic liver disease, Journal of Gastroenterology and Hepatology, **20**, 1102–1108 **(2005)**.

[18] A.Gnasso, C.Motti, C.Irace; Genetic variation in human stromelysin gene promoter and common carotid geometry in healthy male subjects, ArteriosclerThromb.Vasc.Biol., **20**, 1600-1605 **(2000)**.

[19] I.Nelissen, K.Vandenbroeck, P.Fiten; Polymorphism analysis suggests that the gelatinase B gene is not a susceptibility factor for multiple sclerosis, J.Neuroimmunol., **105**, 58-63 **(2000)**.

[20] R.Levesque; SPSS Programming and Data Management, A sGuide for SPSS and SAS Users, 4th Edition, SPSS Inc., Chicago II, **(2007)**

[21] P.A.Bradbury, R.Zhai, J.Hopkins, M.H.Kulke, R.S.Heist, S.Singh, W.Zhou, C.Ma, W.Xu, K.Asomaning, M.Ter-Minassian, Z.Wang, L.Su, D.C.Christiani, G.Liu; Matrix metalloproteinase 1, 3 and 12 polymorphisms and esophageal adenocarcinoma risk and prognosis, Carcinogenesis, **30**, 793-798 **(2009)**.

[22] Y.Zhai, W.Qiu, X.J.Dong, X.M.Zhang, W.M.Xie, H.X.Zhang, X.Y.Yuan, G.Q.Zhou, F.C.He; Functional polymorphisms in the promoters of MMP-1, MMP-2, MMP-3, MMP-9, MMP-12 and MMP-13 are not associated with hepatocellular carcinoma risk, Gut, **56**, 445-447 **(2007)**.

[23] M.Früh, W.Zhou, R.Zhai, L.Su, R.S.Heist, J.C.Wain, N.S.Nishioka, T.J.Lynch, F.A.Shepherd, D.C.Christiani, G.Liu; Polymorphisms of inflammatory and metalloproteinase genes, Helicobacter pylori infection and the risk of oesophageal adenocarcinoma, Br.J.Cancer, **98**, 689-692 **(2008)**.

[24] P.Xia, D.M.Chang, C.X.Dang, L.Meng, H.Xue, Y.Liu; Association between the -1562 C/T polymorphism in the MMP-9 promoter and phenotype of esophageal squamous cell carcinoma in northern Chinese population., Acad.J.Xi'an Jiaotong Univ., **22**, 39-43 **(2010)**.