

## Matrix Metalloproteinase (MMP) -1, -2, -3 and -9 Promoter Polymorphisms in Colorectal Cancer

NILS ELANDER, PETER SÖDERKVIST and KARIN FRANSÉN

Division of Cell Biology, Department of Biomedicine and Surgery,  
Faculty of Health Sciences, SE-581 85 Linköping, Sweden

**Abstract.** *Background:* Matrix metalloproteinases (MMPs) are a group of matrix-degrading proteins implicated in several pathological processes, e.g., invasion and metastasis in malignant diseases such as colorectal cancer (CRC). *Materials and Methods:* One hundred and twenty-seven CRC patients and 208 controls were genotyped for MMP-1, -2, -3 and -9 promoter polymorphisms. The genotyping was performed with PCR/primer-extension/DHPLC or PCR/RFLP. *Results:* The MMP-1 2G allele was significantly associated with CRC ( $p=0.037$ ). No significant association between CRC and MMP-2, -3 or -9 polymorphisms was evident. The analysis of polymorphisms in the clinicopathological subgroups displayed no significant associations. *Conclusion:* The MMP-1 promoter polymorphism seems to affect the susceptibility to CRC, while MMP-2, -3 and -9 polymorphisms appear less likely to have any impact on CRC.

Cancer development is characterized by defects in cellular mechanisms controlling cell growth, e.g., apoptosis, cell cycle-regulating signals, angiogenesis, metastasis and invasion (1). Several investigations have indicated that the matrix metalloproteinases (MMPs), a group of matrix-degrading proteins, are essentially involved in cancer pathobiology (e.g., invasion and metastasis), not least in the case of colorectal cancer (CRC) (2-6). The promoters of many MMP genes contain binding sites and are regulated by transcription factors, such as those of the Fos, Jun and Ets families (7). Physical contact between a transcription factor and its binding site may be influenced by polymorphisms in the promoter, which may result in altered

gene expression (7). Functional polymorphisms in MMP promoter regions have been reported to be associated with several diseases including cancer (7). A single nucleotide polymorphism in the MMP-1 promoter at -1607 has been detected and gives rise to 1G or 2G alleles (8). The 2G allele is associated with augmented transcription of MMP-1 (8) and seems to influence the risk of CRC (9,10).

In addition, the MMP-3 promoter region contains a polymorphism at -1171, creating a 5A or a 6A allele (11). The 6A allele is associated with reduced gene expression (12) and Hinoda *et al.* indicated that the 6A/6A genotype was involved in CRC (9). Furthermore, a C->T substitution polymorphism at -1306 in the MMP-2 promoter seems to lower the MMP-2 promoter activity (13), with possible implications in the development of lung cancer, gastric cardia adenocarcinoma and CRC (14-16).

Finally, the MMP-9 promoter region also contains a C->T substitution at -1562, with the T allele related to a higher promoter activity (17). Implications in cancer invasiveness and progression have been suggested, e.g., in the case of gastric cancer (18).

The present investigation aimed to explore a possible association between these 4 polymorphisms (MMP-1 -1607 1G/2G, MMP-2 -1306 C/T, MMP-3 -1171 5A/6A and MMP-9 -1562 C/T) and CRC susceptibility in a Swedish population. Possible associations with clinicopathological parameters such as age, gender, Dukes' stage, ulcerative/polypoid phenotype and localization were also investigated.

### Materials and Methods

*Patients and tissue collection.* Blood samples were obtained from 127 CRC patients diagnosed at the Ryhov County Hospital, Jönköping, Sweden. The samples were snap-frozen and stored at -70°C until DNA purification.

All patients underwent surgery and the tumor tissue was saved and examined by an experienced pathologist. The age of the patients ranged from 26 to 93 years with a median of 74 years. Sixty-seven of the patients were females and 60 were males. The tumor stages were described according to the Dukes' staging

*Correspondence to:* Nils Elander, Division of Cell Biology, Floor 9, Department of Biomedicine and Surgery, Faculty of Health Sciences, SE-581 85 Linköping, Sweden. Tel: +46 (0)13 - 222663, Fax: +46 (0)13 - 221718, e-mail: nilel463@student.liu.se

*Key Words:* MMP-1, MMP-2, MMP-3, MMP-9, promoter, polymorphism, colorectal cancer.

Table I. Primer sequences and PCR conditions.

Polymorphism	[MgCl <sub>2</sub> ]	Primer sequences	Temp. <sup>1</sup>
MMP-1 -1607 1G/2G	2.5 mM	GTGAGAATGTCTTCCCATTCTTCT <sup>2</sup> GGATTGATTGAGATAAGTCATAGC <sup>3</sup> GTAGTTAAATAATTAGAAAG <sup>4</sup>	58.0 °C
MMP-2 -1306 C/T	2.0 mM	CTTCCTAGGCTGGTCCTTAC <sup>2</sup> AGACCTGAAGAGCTAACAGACG <sup>3</sup> ATATTCCCCACCCAGCACTC <sup>4</sup>	55.0 °C
MMP-3 -1171 5A/6A	3.0 mM	GATTACAGACATGGGTACA <sup>2</sup> (20) TTCAATCAGGACAAGACGAAGTT <sup>3</sup> (20)	50.0 °C
MMP-9 -1562 C/T	2.0 mM	CAACGTAGTGAAACCCATCTCT <sup>2</sup> (25) TCCAGGCCAATTATCACACTTAT <sup>3</sup> (25) GTAGCTGGTATTATAGGC <sup>4</sup>	60.0 °C

<sup>1</sup>Annealing temperature for PCR primers.<sup>2</sup>Forward PCR primer.<sup>3</sup>Reverse PCR primer.<sup>4</sup>Primer extension primer.

system; 18 tumors were in Dukes' stage A, 59 in B, 42 in C and 8 in D. Seventy-seven of the tumors displayed an ulcerative phenotype, 37 a polypoid phenotype and 5 a mixed phenotype. Eight of the tumors were not classified due to a polypoid/ulcerative phenotype. Sixty-seven tumors were colonic (cecum 11, ascending colon 23, transverse colon 4, descending colon 1, sigmoid colon 28) and 55 rectal. No information about localization was available in 5 cases. The control group consisted of 208 randomly-collected healthy individuals from the same geographical area. The investigation was approved by the Research Ethical Committee of the Faculty of Health Sciences, Linköping, Sweden (Dnr. 98113).

**DNA isolation.** Normal genomic DNA from peripheral blood was extracted with a Wizard® Genomic DNA Purification Kit, according to the manufacturer's recommendations (Promega, Madison, WI, USA).

**PCR amplification.** The PCR reactions were performed in a volume of 20 µl with the following conditions: 75 mM Tris-HCl (pH 9.0), 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.01% Tween 20, MgCl<sub>2</sub> (for concentrations see Table I), 0.2 mM of each dNTP, 1 µM forward primer (Table I), 1 µM reverse primer (Table I), 10-20 ng of genomic DNA and 1 U Taq polymerase (10 U/µl, Invitrogen, Stockholm, Sweden). The amplification reactions were followed by visualization with ethidium bromide and UV-light.

**Genotyping of the MMP-1, -2 and -9 polymorphisms.** Purification of the PCR products was performed according to the supplier's recommendations; 5 µl of PCR product was mixed with 2 ml of ExoSAP-IT® (Amersham Biosciences AB, Uppsala, Sweden) and incubated at 37°C for 15 min, followed by 80°C for 15 min.

The genotyping was performed with primer extension and denaturing high performance liquid chromatography (DHPLC), according to Hoogendorn *et al.* (19). Twenty µl of reaction volume were used, containing 0.05 mM of a dideoxyNTP (ddGTP for

MMP-1, ddTTP for MMP-2, ddATP for MMP-9, respectively), 0.05 mM of each remaining dNTP, 0.5 U Thermosequenase™ (Amersham Biosciences), 2 µl of Thermosequenase™ buffer (Amersham Biosciences), 0.6 µM of primer extension primer (Table I) and 7 µl of template. The extension reaction was subsequently performed; an initial denaturation step at 94°C for 2 min followed by 50 cycles of 94°C for 5 sec, 44°C for 5 sec and 60°C for 5 sec. DHPLC was carried out using the Wave® DNA Fragment Analysis System (Transgenomic Inc., Omaha, Nebraska, USA). The length of the products was estimated with an UV detector and presented as chromatograms.

**Genotyping of the MMP-3 polymorphism.** Restriction endonuclease digestion was performed according to Dunleavy *et al.* (20). However, instead of using the restriction endonuclease *XmnI*, the isoschizomer *PdmI* (Fermentas, St. Leon-Rot, Germany) was employed. The PCR reverse primer contained 3 mismatched nucleotides close to the 5A/6A polymorphism, creating a recognition site for the endonuclease.

Thirteen µl of PCR product solution were mixed with 1.5 ml of Tango™ buffer (Fermentas) and 10 U of *PdmI* (10 U/µl, Fermentas) and were incubated at 37°C for 16 h. Thereafter, 13 µl of the digest solution was mixed with 2 µl of loading dye. The electrophoresis was performed on a 3% NuSieve® (Cambrex Bioscience Rockland Inc., Rockland, ME, USA) 1% Agarose (Invitrogen) gel at 120 V for 60 min. The gel was stained with ethidium bromide and the DNA fragments were visualized under UV-light.

**DNA sequencing.** To confirm the genotyping results, random samples for all polymorphisms investigated were sequenced with the MegaBACE 500 DNA Analysis Systems (Amersham Biosciences), following the manufacturer's recommendations.

**Statistical analyses.** The χ<sup>2</sup>-test with Yates correction and Fisher's exact test were performed with the SPSS 12.0.1 statistical software

Table II. Power estimations.

	MMP-1 -1607	MMP-2 -1306	MMP-3 -1171	MMP-9 -1562
Allele frequency <sup>1</sup>	49.0% (2G allele)	27.5% (T allele)	48.0% (6A allele)	13.0% (T allele)
Power <sup>2</sup>	85.7%	83.2%	85.9%	64.6%

<sup>1</sup>Allele frequencies were based on previous investigations in healthy Caucasian populations (23, 26).

<sup>2</sup>Power was calculated at [http://calculators.stat.ucla.edu/powercalc/] using a two-sided  $\chi^2$  test. The relative risk was set at 2.0.

Table III. Genotype and allele frequencies of patients and controls.

Polymorphism	Genotype/Aallele	Patients (%)	Controls (%)	P <sup>1</sup>	OR <sup>1</sup>	95% CI <sup>1</sup>
MMP-1 -1607 1G/2G	1G/1G	23 (18.1)	55 (26.4)		(1.00)	
	1G/2G	61 (48.0)	101 (48.6)			
	2G/2G	43 (33.9)	52 (25.0)	0.105	1.54	0.92-2.56
	1G allele	107 (42.1)	211 (50.7)			
	2G allele	147 (57.9)	205 (49.3)	0.037	1.41	1.02-1.96
MMP-2 -1306 C/T	T/T	9 (7.1)	10 (4.8)		(1.00)	
	C/T	49 (38.6)	89 (42.8)			
	C/C	69 (54.3)	109 (52.4)	0.818	1.08	0.68-1.72
	T allele	67 (26.4)	109 (26.2)			
	C allele	187 (73.6)	307 (73.8)	1.000	0.99	0.69-1.44
MMP-3 -1171 5A/6A	5A/5A	37 (29.1)	48 (23.1)		(1.00)	
	5A/6A	52 (40.9)	115 (55.3)			
	6A/6A	38 (29.9)	45 (21.6)	0.115	1.55	0.91-2.64
	5A allele	126 (49.6)	211 (50.7)			
	6A allele	128 (50.4)	205 (49.3)	0.841	1.05	0.76-1.45
MMP-9 -1562 C/T	C/C	97 (76.4)	165 (79.3)		(1.00)	
	C/T	30 (23.6)	41 (19.7)			
	T/T	0 (0.0)	2 (1.0)	0.618	1.19	0.68-2.08
	C allele	224 (88.2)	371 (89.2)			
	T allele	30 (11.8)	45 (10.8)	0.788	1.10	0.66-1.85

<sup>1</sup>Association was analyzed with  $\chi^2$  with the Yates correction. P are values for MMP-1 2G/2G relative to 1G/2G+1G/1G, MMP-2 C/C relative to C/T+T/T, MMP-3 6A/6A relative to 5A/6A+5A/5A and MMP-9 T/T+C/T relative to the C/C genotypes, respectively. OR=Odds ratio. CI=Confidence interval.

package (SPSS Inc., Chicago, IL, USA). The odds ratio and 95% confidence interval were also analyzed when appropriate. The Hardy-Weinberg (H-W) equilibrium was tested for all polymorphisms in the control samples and no deviations were found. Furthermore, the power was calculated at [http://calculators.stat.ucla.edu/powercalc/] for all polymorphisms investigated, using a two-sided  $\chi^2$ -test with the relative risk set at 2.0 (Table II). The statistical significance level was set at  $p<0.05$ .

## Results

**MMP-1 -1607 1G/2G polymorphism.** The 2G allele was significantly more frequent among patients than controls

( $p=0.037$ , OR=1.41, 95% CI 1.02-1.96) (Table III). No significant differences were found when comparing the genotype distributions among patients and controls (Table III), nor was any association with the 2G allele or the 2G/2G genotype and clinicopathological parameters (age, gender, Dukes' stage, ulcerative/polypoid phenotype and localization) evident (data not shown).

**MMP-2 -1306 C/T, MMP-3 -1171 5A/6A and MMP-9 -1562 C/T polymorphisms.** The genotype- and allele frequencies were analyzed in the patient and control populations (Table III). No significant associations were found regarding CRC

and the allele distribution of MMP-2 -1306 C/T ( $p=1.000$ , OR= 0.99, 95% CI 0.69-1.44), MMP-3 -1171 5A/6A ( $p=0.841$ , OR=1.05, 95% CI 0.76-1.45) or MMP-9 -1562 C/T ( $p=0.788$ , OR=1.10, 95% CI 0.66-1.85). Furthermore, a comparison of genotype distributions among patients and controls regarding the MMP-2, -3 and -9 polymorphisms revealed no significant differences (Table III). Finally, no association was evident between the allele/genotype distribution of the MMP-2, -3 and -9 polymorphisms and clinicopathological parameters of CRC (data not shown).

## Discussion

The present investigation was aimed at studying a possible correlation between 4 functional MMP promoter polymorphisms and CRC. The clinicopathological data of the patients were also included in the analyses.

Several previous studies had found overexpression of MMP-1 and -3 in CRC, as well as their importance for disease progression (2-4). The MMP-1 -1607 2G allele was found to increase the MMP-1 gene transcription (8). In the present study, a significantly higher frequency of the 2G allele was found among CRC patients ( $p=0.037$ , OR=1.41, 95% CI 1.02-1.96), which is in agreement with the results of previous investigations (9,10). Thus, there seems to be a connection between the MMP-1-1607 promoter polymorphism and CRC susceptibility. However, no significant association between the 2G/2G genotype and CRC susceptibility, although indicated in previous studies (9,10), was found. In addition, no implication of the polymorphism in the clinicopathological parameters (age, gender, Dukes' stage, ulcerative/polypoid phenotype and localization) of CRC was evident in the present investigation.

Concerning the MMP-3 -1171 5A/6A polymorphism, no associations with CRC susceptibility or with the clinicopathological parameters were found. The 6A allele of the polymorphism seems to lower the MMP-3 gene expression level (12). Considering this, the results published by Hinoda *et al.*, showing a higher frequency of the 6A/6A genotype among CRC patients (9), were surprising. However, one other study did not find this correlation, thus supporting our results (10). Furthermore, larger sample sizes were included than in the previous investigations and the power in our study was approximately 86% (Table II), which indicates that the MMP-3 -1171 6A allele probably has no impact on CRC development. However, the MMP-3 -1171 6A allele, as well as the MMP-1 -1607 2G allele, may have protective roles in the development of some cancers, *e.g.*, squamous cell carcinomas of head and neck (21), which may indicate different effects of MMP-1 and -3 promoter polymorphisms among cancers.

With regard to MMP-2 and -9, a role in the metastasis cascade has been suggested due to their collagen IV degrading capability. In addition, other possible functions

related to carcinogenesis have been indicated, *e.g.*, an impact on cell differentiation, apoptosis, angiogenesis, immune surveillance and cancer cell growth (22). Previous investigations have indicated increased expressions of MMP-2 and -9 in CRC tumors (2, 4-6), which may be a result of promoter polymorphisms influencing the MMP-2 and -9 expression levels in CRC development. However, no significant associations were found with the highly activity C allele of the MMP-2 -1306 C/T polymorphism and CRC, or with clinicopathological parameters. These findings are not in accordance with the results of Xu *et al.*, who showed a correlation between the C/C genotype and CRC susceptibility and serosa/adventitia involvement (16). However, our investigation included larger sample sizes and had a higher power (83.2%, Table II).

Moreover, there are indications for an association with the C/C genotype and lung cancer and gastric cardia adenocarcinoma (14, 15). The T/T genotype was found to be associated with smaller tumors and lower estrogen receptor levels in one study on breast cancer (23). However, a dual effect of the T/T genotype in breast cancer prognosis was found, depending on the estrogen receptor status (23). Thus, the role of the MMP-2 -1306 C/T polymorphism in cancer seems to be complex and might differ among cancers.

No associations were found between susceptibility for or clinicopathological parameters of CRC and the functional MMP-9 -1562 C/T polymorphism, in contrast to findings of previous studies on other cancers. The T allele was found to be associated with positive prognostic features in breast cancer (23), but it seems to be related to the invasiveness of gastric cancer (18). Furthermore, no correlations between the polymorphism and non-small cell lung carcinoma (NSCLC) or lymphatic metastases in NSCLC were found in a previous study (24), either indicating the different effects of the polymorphism in different cancers or chance associations.

In conclusion, the MMP-1 -1607 1G/2G polymorphism seems to influence the development of CRC, whereas the MMP-2 -1306 C/T, MMP-3 -1171 5A/6A and MMP-9 -1562 C/T polymorphisms do not.

## Acknowledgements

We greatly appreciate the assistance of Annette Molbaek in the MegaBACE genotyping, Alf Kastbom in the primer extension/DHPLC and Dr Jan Dimberg, Dr Anders Hugander and Eva Georgsson in collecting the patient material and normal blood DNA samples. This study was supported by grants from the Swedish Cancer Foundation.

## References

- 1 Hanahan D and Weinberg RA: The hallmarks of cancer. *Cell* 100: 57-70, 2000.

- 2 Zucker S and Vacirca J: Role of matrix metalloproteinases (MMPs) in colorectal cancer. *Cancer Metastasis Rev* 23: 101-117, 2004.
- 3 Murray GI, Duncan ME, O'Neil P, Melvin WT and Fothergill JE: Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. *Nat Med* 2: 461-462, 1996.
- 4 Baker EA, Bergin FG and Leaper DJ: Matrix metalloproteinases, their tissue inhibitors and colorectal cancer staging. *Br J Surg* 87: 1215-1221, 2000.
- 5 Parsons SL, Watson SA, Collins HM, Griffin NR, Clarke PA and Steele RJ: Gelatinase (MMP-2 and -9) expression in gastrointestinal malignancy. *Br J Cancer* 78: 1495-1502, 1998.
- 6 Levy AT, Cioce V, Sobel ME, Garbisa WF, Liotta LA and Stettler-Stevenson WG: Increased expression of the Mr 72,000 type IV collagenase in human colonic adenocarcinoma. *Cancer Res* 51: 439-444, 1991.
- 7 Ye S: Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. *Matrix Biol* 19: 623-629, 2000.
- 8 Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ and Brinckerhoff CE: A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res* 58: 5321-5325, 1998.
- 9 Hinoda Y, Okayama N, Takano N, Fujimura K, Suehiro Y, Hamanaka Y, Hazama S, Kitamura Y, Kamatani N and Oka M: Association of functional polymorphisms of matrix metalloproteinase (MMP)-1 and MMP-3 genes with colorectal cancer. *Int J Cancer* 102: 526-529, 2002.
- 10 Ghilardi G, Biondi ML, Mangoni J, Leviti S, DeMonti M, Guagnellini E and Scorza R: Matrix metalloproteinase-1 promoter polymorphism 1G/2G is correlated with colorectal cancer invasiveness. *Clin Cancer Res* 7: 2344-2346, 2001.
- 11 Ye S, Watts GF, Mandalia S, Humphries SE and Henney AM: Preliminary report: genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis. *Br Heart J* 73: 209-215, 1995.
- 12 Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE and Henney AM: Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J Biol Chem* 271: 13055-13060, 1996.
- 13 Price S, Greaves D and Watkins H: Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene. *J Biol Chem* 276: 7549-7558, 2001.
- 14 Yu C, Pan K, Xing D, Liang G, Tan W, Zhang L and Lin D: Correlation between a single nucleotide polymorphism in the matrix metalloproteinase-2 promoter and risk of lung cancer. *Cancer Res* 62: 6430-6433, 2002.
- 15 Miao X, Yu C, Tan W, Xiong P, Liang G, Lu W and Lin D: A functional polymorphism in the matrix metalloproteinase-2 gene promoter (-1306C/T) is associated with risk of development but not metastasis of gastric cardia adenocarcinoma. *Cancer Res* 63: 3987-3990, 2003.
- 16 Xu E, Maode L, Lv B, Xing X, Huang Q and Xia X: A single nucleotide polymorphism in the matrix metalloproteinase-2 promoter is associated with colorectal cancer. *Biochem Biophys Res Commun* 324: 999-1003, 2004.
- 17 Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A, Arveiler D, Luc G, Cambien F, Hamsten A, Watkins H and Henney AM: Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 99: 1788-1794, 1999.
- 18 Matsumura S, Oue N, Nakayama H, Kitadai Y, Yoshida K, Yamaguchi Y, Imai K, Nakachi K, Matsusaki K, Chayama K and Yasui W: A single nucleotide polymorphism in the MMP-9 promoter affects tumor progression and invasive phenotype of gastric cancer. *J Cancer Res Clin Oncol* 131: 19-25, 2005.
- 19 Hoogendorn B, Norton N, Kirov G, Williams N, Hamshere ML, Spurlock G, Austin J, Stephens MK, Buckland PR, Own MJ and O'Donovan MC: Cheap, accurate and rapid allele frequency estimation of single nucleotide polymorphisms by primer extension and DHPLC in DNA pools. *Hum Genet* 107: 488-493, 2000.
- 20 Dunleavy L, Beyzade S and Ye S: Rapid genotype analysis of the stromelysin gene 5A/6A polymorphism. *Atherosclerosis* 151: 587-589, 2000.
- 21 Zinzindohoue F, Blons H, Hans S, Loriot MA, Houllier AM, Brasnu D, Laccourreye O, Tregouet DA, Stucker I and Laurent-Puig P: Single nucleotide polymorphisms in MMP1 and MMP3 gene promoters as risk factor in head and squamous cell carcinoma. *Anticancer Res* 24: 2021-2026, 2004.
- 22 Mook ORF, Frederiks WM and van Noorden CJF: The role of gelatinases in colorectal cancer progression and metastasis. *Biochim Biophys Acta* 1705: 69-89, 2004.
- 23 Grieu F, Li WQ and Iacopetta B: Genetic polymorphisms in the MMP-2 and MMP-9 genes and breast cancer phenotype. *Breast Cancer Res Treat* 88: 197-204, 2004.
- 24 Wang Y, Fang S, Wei L, Wang R, Jin X, Wen D, Li Y, Guo W, Wang N and Zhang J: No association between the C-1562T polymorphism in the promoter of matrix metalloproteinase-9 gene and non-small cell lung carcinoma. *Lung Cancer* 49: 155-161, 2005.
- 25 Jones GT, Phillips VL, Harris EL, Rossaak JI and van Rij AM: Functional matrix metalloproteinase-9 polymorphism (C-1562T) associated with abdominal aortic aneurysm. *J Vasc Surg* 38: 1363-1367, 2003.
- 26 Wiencke K, Louka AS, Spurkland A, Vatn M, Schrumpf E and Boberg KM: Association of matrix metalloproteinase-1 and -3 promoter polymorphisms with clinical subsets of Norwegian primary sclerosing cholangitis patients. *J Hepatol* 41: 209-214, 2004.

*Received September 28, 2005**Revised November 30, 2005**Accepted December 2, 2005*