Matrix metalloproteinases in inflammatory bowel disease : expression, regulation and clinical relevance

Meijer, M.J.W.

Chapter 7

Matrix Metalloproteinases and their Tissue Inhibitors as Prognostic Indicators for Diagnostic and Surgical Recurrence in Crohn's Disease

Short title: Recurrence in CD

Martin J.W. Meijer
Marij A.C. Mieremet-Ooms
Cornelis F.M. Sier
Ruud A. van Hogezand
Cornelis B.H.W. Lamers
Daniel W. Hommes
Hein W. Verspaget

Department of Gastroenterology and Hepatology
Leiden University Medical Center
The Netherlands

This Study was published in Inflamm Bowel Dis 2009; 15(1): 84-92

Abstract

Background/aims. Recurrence of disease after surgically induced remission constitutes a major and largely unpredictable problem in Crohn's disease (CD). Matrix metalloproteinases (MMP) and the tissue inhibitors of metalloproteinases (TIMP) are involved in the (etio)pathogenesis of CD and may thereby also affect postsurgical outcome. We studied the predictive value of 1) allelic composition at MMP, TIMP, and TNF-α single nucleotide polymorphism loci, and 2) MMP and TIMP intestinal protein levels relative to important clinical variables for recurrence of CD after resection of diseased bowel.

Methods. From 87 CD patients with a full medical record, surgically resected tissue was homogenized and analyzed for single nucleotide polymorphism (SNP) genotype and MMP-TIMP protein levels. The prognostic value of these parameters was determined using the uni- and multivariate Cox proportional hazards analyses.

Results. The T-allele at TIMP-1 SNP +372 T/C was found to be associated with an increased risk for surgical recurrence. Higher levels of TIMP-1, TIMP-2, and MMP-9 in noninflamed CD tissue, but not in inflamed tissue, and negative smoking status independently protected against diagnostic and/or surgical recurrence.

Conclusions. The TIMP-1 SNP +372 T allele with an increased risk of recurrence is in line with our previous results demonstrating increased CD susceptibility and low TIMP-1 protein expression associated with this allele. High TIMP and MMP-9 levels in non-inflamed tissue are predictive of a favorable disease recurrence in CD. The contribution of MMP-9 and TIMPs to disease recurrence appears not to be mediated by smoking status, since no correlation with this parameter could be demonstrated.

Introduction

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. Although the etiology is largely unknown, current understanding suggests that the sustained immune infiltration manifests as an aberrant reaction to luminal bacteria in genetically susceptible individuals.[1] It is characterized by chronic, segmental, and transmural inflammation of the bowel wall, particularly ileocolonic, treated with immunomodulating drugs to induce clinical remission. Despite advances in medical therapy, still a large proportion of CD patients will eventually require resection of diseased bowel, with an estimated cumulative probability during the first decade after diagnosis ranging between 30% and 60%.[2,3] One year after resection, endoscopic and clinical recurrence occurs in 70% and 20% of patients, respectively.[4] The cumulative second surgical recurrence rate amounts to 35.9% 10 years after first bowel resection, with a small but substantial group at hazard for even more subsequent resections (third surgical recurrence rate 39.8% 5 years after the second bowel resection).[5] Several studies focused on identifying predictive variables for postsurgical recurrence, including age at onset of disease, age at time of surgery, duration of disease before initial operation, type of surgery (i.e., laparoscopic versus open conventional), disease localization, gender, histology, etc., with sometimes inconclusive or even contrasting results.[6-14] Smoking behavior, however, was consistently found to adversely affect disease prognosis, both in medical and surgically induced remission.[15] Identification and selection of patients prone to recurrence would enhance the effectiveness of adjuvant medical therapy with azathioprine, ornidazole, or infliximab,[16-18] thus limiting costs and drug-related side effects.

Matrix metalloproteinases (MMP) constitute a group of neutral endoproteinases, collectively capable of cleaving various intestinal matrix proteins, with proteolytic activity tightly regulated by their natural inhibitors, the tissue inhibitors of metalloproteinases (TIMPs).[19] In inflamed CD and ulcerative colitis tissue, several MMPs, including MMP-1, -3, -9, and -12 relative to TIMP-1 and -2 are overexpressed[20-27] leading to an increase in net MMP proteolytic activity.[28,29] Even in the macroscopically normal proximal resection margin, an upregulation of MMP-3 has been observed.[30] Part of this proteolytic phenotype seen in

inflammatory bowel diseases (IBDs) may be attributed to increased TNF-α signaling.[31,32] In the genes coding for MMPs, TIMPs, and TNF-α, single nucleotide polymorphisms (SNPs) have been described, and allelic composition at these SNPs may determine protein expression. For instance, MMP-1 protein expression is upregulated in cells carrying 2 copies of the 2G allele at MMP-1 promoter –1607.[33] In previous reports, the 5T and T allele at SNP MMP-3 –1613 5T/6T and TIMP-1 +372 T/C, respectively, were found to be associated with CD,[34,35] while contradictory results were obtained for the –308 G/A SNP at the TNF-α gene.[36,37] Therefore, CD patients with different genetic MMP, TIMP, or TNF-α constitution may express different levels of corresponding proteins and follow a different clinical course after surgically induced remission. Previously, we determined allelic composition at MMP-1, -2, -3, -9, TIMP-1, -2, and TNF-α SNP loci in CD patients and also measured MMP and TIMP protein levels in surgically resected inflamed and macroscopically normal tissue.[29,34] Here we relate the CD SNP allelic distribution and tissue protein expression to postsurgical recurrence data in a multivariate model including major clinical variables.

Materials and methods

Patient population

For this retrospective study, 87 predominantly Dutch Caucasian CD patients with a full medical record and prospectively collected resection specimens were included. Diagnosis of CD was established by routine endoscopical, radiological, and histological examination. Surgical intestinal resections with anastomosis of macroscopically normal bowel were performed in the period 1984–2000, median follow-up after resection was 8.4 (0.9–19.1) years. Location of disease was often ileocecal (n = 66), with 8 and 13 patients undergoing small bowel resection or (sub)total colectomy, respectively. Indication for surgery was stenotic narrowed tissue, medical refractory disease, or perforation (n = 36, 50, and 1, respectively). Diagnostic recurrence was defined as a rise in clinical symptoms with endoscopy and/or radiology-confirmed inflammation of previously unaffected bowel. Surgical recurrence was defined as resection of diseased tissue macroscopically normal at

previous surgery.

Genotype and protein determination

Resected tissue was collected at the Department of Pathology, LUMC, and stored at -70°C until analysis. Mucosa was homogenized with a Turrax device, genomic DNA was isolated according to the salting out method[38] and reconstituted to 10 ng/μL in 0.01 M Tris / 0.1 mM EDTA, pH 7.5. Allelic composition at the SNP loci studied was determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) (MMP-1, -3, -9, TIMP-1, -2, TNF-α) or tetra primer ARMS PCR (MMP-2), as described previously.[39,40] In 1 patient DNA isolation failed and the genotype could not be determined. From 85 and 76 patients, respectively, inflamed and noninflamed mucosa was obtained and homogenized in 1 mL 0.1 M Tris-HCl, 0.1% Tween 80, pH 7.5 per 60 mg tissue using a Potter device (B Braun, Germany). Antigen protein levels of MMP-2 and MMP-9 were measured in appropriately diluted homogenates by our in-house enzyme-linked immunosorbent assays (ELISAs), as described before,[41] while TIMP-1 and -2 protein were measured by commercially available ELISAs from R&D Systems (Minneapolis, MN), according to the manufacturer's instructions. All antigen levels were corrected for total protein amount, as determined by the method of Lowry *et al*.[42] The protein levels of MMP-1, MMP-3, and TNF-α were either not assessed or at a too low number for adequate analyses.

Statistical analysis

Differences between subgroups were calculated using the Mann–Whitney U-test or Pearson Chi Square test as indicated. For disease follow-up analyses the clinical parameters were dichotomized as follows: smoking habit after surgical resection (yes versus no), gender (male versus female), medication before surgical resection (mild: nothing or mesalazine versus strong: corticosteroids and/or immunomodulatory drugs w/wo mesalazine), age at surgery (≤ 27.6 versus >27.6 years), age at onset of disease (≤ 16.65 years versus >16.65 years), number of former resections (0 versus >0), and time lag between onset of disease and surgical resection (≤ 11.89 versus >11.89 years). Cutoff values for continuous

clinical variables, MMP, and TIMP levels were optimized using receiver operator characteristic (ROC) analyses. Multivariate analyses were performed with the Cox proportional hazards method by separately adding variables to the dichotomized clinical parameters. Survival curves were constructed using the method of Kaplan and Meier including log-rank tests. Differences were considered significant when $P < 0.05$. Analyses were performed using the SPSS statistical package (Release 11.0, Chicago, IL).

Results

Clinical parameters and recurrence

In the univariate Cox analyses the 49 patients with a positive smoking status were at increased risk for developing diagnostic recurrence compared to the 38 non-smokers (hazard ratio [HR] 1.846, $P < 0.05$; Table 1). Median time of reaching the diagnostic recurrence endpoint dropped from 8.2 to 2.8 years ($P = 0.02$; Fig. 1A). Young age at surgery and at onset of disease (27.6 and 16.65 years, respectively) also exposed patients to earlier diagnostic recurrence. Gender, medication 1 month prior to surgery, number of previous resections, and time lag between onset of disease and surgery had no effect. In multivariate analyses including all clinical variables, only smoking maintained its prognostic value (HR 2.002, $P < 0.02$). Smoking, but not the other clinical parameters, also significantly increased the risk for surgical recurrence (HR 4.033, $P = 0.001$), with the median time lag for re-resection dropping from 17.2 to 9.3 years ($P = 0.0005$; Fig. 1B) and proved an independent prognostic indicator with a similar hazard ratio in the multivariate analyses. However, smoking was not associated with MMP-TIMP genotype or tissue protein levels (Suppl. Table X), providing a rationale for further hazard analyses including these parameters.

Genotype and tissue MMP-TIMP protein levels versus recurrence

The allelic distribution at MMP, TIMP, and TNF-α SNP loci was not associated with diagnostic recurrence (Table 2). In univariate analyses, only the C allele at SNP TIMP-1 +372 T/C was found to be protective against surgical recurrence. Patients

Table 1. Cox regression analyses of selected clinical variables in relation to diagnostic (A) and surgical (B) recurrence after resection of diseased bowel in 87 CD Patients.

A) Diagnostic recurrence

		Univariate			Multivariate		
Parameter	*N*	HR	95% CI	*P*	HR	95% CI	*P*
Smoking			1.091-				
Negative vs positive	38/49	1.846	3.124	0.022	2.002	1.144-3.504	0.015
Gender			0.857-				
Male vs female	35/52	1.445	2.436	NS	1.302	0.721-2.351	NS
Medication*			0.858-				
Mild vs strong	38/49	1.441	2.419	NS	1.146	0.626-2.100	NS
Age at surgery			0.315-				
≤ 27.6 vs > 27.6 yrs	25/62	0.534	0.904	0.020	0.648	0.298-1.409	NS
Age at onset of disease			0.313-				
≤ 16.65 vs > 16.65 yrs	22/65	0.537	0.923	0.024	0.587	0.300-1.150	NS
Nr of previous resections			0.583-				
0 vs >0	52/35	0.975	1.630	NS	1.443	0.774-2.689	NS
Time lag onset of							
disease-resection			0.510-				
≤ vs > median (11.89 yrs)	44/43	0.847	1.409	NS	0.874	0.459-1.664	NS

B) Surgical recurrence

		Univariate			Multivariate		
Parameter	*N*	HR	95% CI	*P*	HR	95% CI	*P*
Smoking			1.733-			1.720-	
Negative vs positive	38/49	4.033	9.385	0.001	4.206	10.286	0.002
Gender			0.894-				
Male vs female	35/52	1.906	4.063	NS	1.664	0.730-3.792	NS
Medication*			0.592-				
Mild vs strong	38/49	1.202	2.438	NS	0.896	0.380-2.115	NS
Age at surgery			0.442-				
≤ 27.6 vs > 27.6 yrs	25/62	0.938	1.989	NS	1.056	0.353-3.156	NS
Age at onset of disease			0.630-				
≤ 16.65 vs > 16.65 yrs	22/65	1.532	3.727	NS	1.256	0.451-3.503	NS
Nr of previous resections			0.459-				
0 vs >0	52/35	0.932	1.894	NS	1.457	0.605-3.512	NS
Time lag onset of							
disease-resection			0.462-				
≤ vs > median (11.89 yrs)	44/43	0.926	1.855	NS	0.719	0.267-1.935	NS

* Medication: mild = nothing or mesalazine, strong = corticosteroids, immunomodulatory drugs w/wo mesalazine one month prior to surgical resection. *N*, number of patients; HR, hazard ratio; CI, confidence interval; *P*, statistical significance.

Table 2. Cox proportional hazard analysis for 86 CD Patients testing allelic distribution at MMP, TIMP and TNF-α SNP loci versus clinical parameters in relation to diagnostic (A) and surgical (B) recurrence after resection of diseased bowel.

A) Diagnostic recurrence

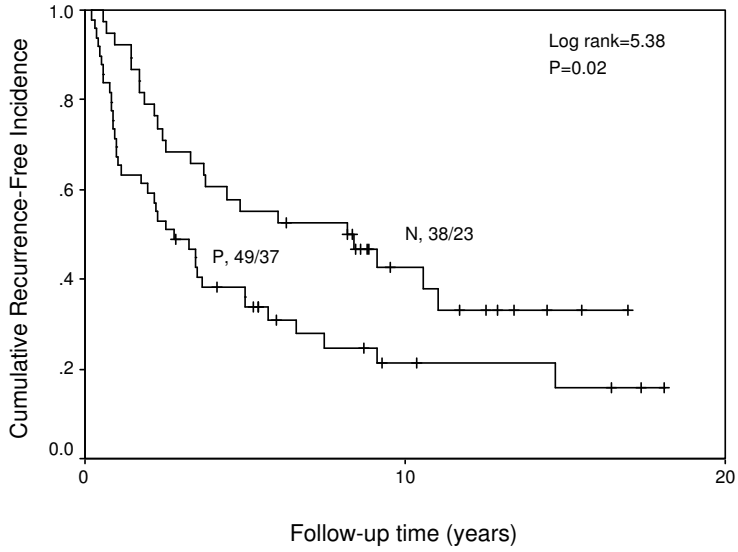
		Univariate			Multivariate		
Parameter	*N*	HR	95% CI	*P*	HR	95% CI	*P*
MMP-1 SNP –1607 1G/2G 1G1G vs 1G2G + 2G2G	26/40+20	1.252	0.712- 2.201	NS	1.282	0.699- 2.352	NS
MMP-2 SNP –1306 C/T CC vs CT + TT	46/35+5	0.841	0.502- 1.407	NS	0.608	0.349- 1.061	NS
MMP-3 SNP –1613 5T/6T 5T5T vs 5T6T + 6T6T	24/40+22	0.980	0.557- 1.724	NS	0.946	0.504- 1.778	NS
MMP-9 SNP –1562 C/T CC vs CT + TT	63/23+0	1.261	0.717- 2.218	NS	1.324	0.712- 2.461	NS
TNF-α SNP –308 G/A GG vs GA + AA	61/21+4	0.863	0.485- 1.537	NS	0.636	0.336- 1.205	NS
TIMP-1 SNP +372 T/C T(T)+CT vs C(C)	41+19/26	0.959	0.548- 1.679	NS	1.224	0.662- 2.265	NS
TIMP-2 SNP +303 G/A GG vs GA + AA	65/20+1	1.251	0.701- 2.233	NS	1.026	0.562- 1.874	NS

B) Surgical recurrence

		Univariate			Multivariate		
Parameter	*N*	HR	95% CI	*P*	HR	95% I	*P*
MMP-1 SNP –1607 1G/2G 1G1G vs 1G2G + 2G2G	26/40+20	0.784	0.379- 1.619	NS	0.925	0.423- 2.022	NS
MMP-2 SNP –1306 C/T CC vs CT + TT	46/35+5	1.177	0.587- 2.364	NS	0.996	0.485- 2.045	NS
MMP-3 SNP –1613 5T/6T 5T5T vs 5T6T + 6T6T	24/40+22	0.698	0.340- 1.433	NS	1.064	0.456- 2.481	NS
MMP-9 SNP –1562 C/T CC vs CT + TT	63/23+0	0.801	0.360- 1.785	NS	0.785	0.338- 1.824	NS
TNF-α SNP –308 G/A GG vs GA + AA	61/21+4	0.575	0.248- 1.333	NS	0.471	0.192- 1.156	NS
TIMP-1 SNP +372 T/C T(T)+CT vs C(C)	41+19/26	0.391	0.160- 0.955	0.039	0.385	0.143- 1.038	NS (*P* = 0.059)
TIMP-2 SNP +303 G/A GG vs GA + AA	65/20+1	1.868	0.876- 3.985	NS	1.558	0.685- 3.545	NS

N, number of patients; HR, hazard ratio; CI, confidence interval; *P*, statistical significance.

A Diagnostic recurrence smoking



B Surgical recurrence smoking

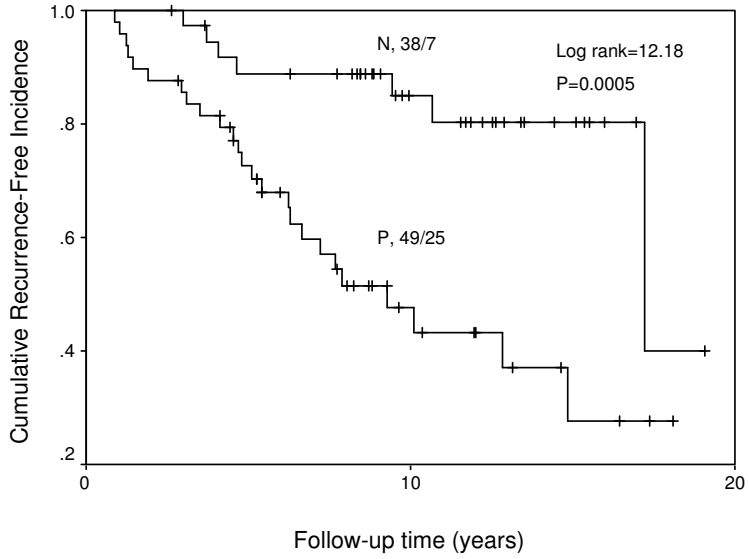


Figure 1. Kaplan–Meier recurrence-free incidence analysis for smoking status. Positive smoking status increased the risk for diagnostic (A) and surgical (B) recurrence, dropping median duration between surgery and diagnostic recurrence from 8.2 to 2.8 years and between surgery and re-resection from 17.2 to 9.3 years, $P < 0.05$. N, P = negative or positive smoking status after surgery, respectively. X/Y = number of patients / number of patients with recurrence.

carrying the T(T) or CT compared to C(C) genotype underwent re-resection 10.7 versus 19.1 years after initial surgery ($P < 0.05$; Fig. 2A). In the univariate analysis we found HR 0.391 ($P < 0.04$) and this association was borderline non-significant in the multivariate analysis ($P = 0.059$). The TIMP-1 gene is X-linked, a survival analysis stratified to gender yielded a similar trend for women (data not shown). High levels of TIMP-1, TIMP-2, and remarkably MMP-9 (2.94, 4.66 and 5.11 ng/mg, respectively) in noninflamed tissue decreased the risk for diagnostic recurrence (Table 3). Of these only MMP-9 was found to be an independent prognostic variable (HR = 0.498, $P < 0.05$). High levels of TIMP-1, TIMP-2, and MMP-9 (2.88, 4.66, and 5.11 ng/mg, respectively) in noninflamed tissue were also found to be protective against surgical recurrence. For instance, the median time lag between surgery and re-resection was 10.7 years in 17 patients with less than or equal to 2.88 ng/mg TIMP-1 in noninflamed tissue while in 59 patients with TIMP-1 levels above this threshold it was 17.2 years ($P = 0.03$; Fig. 2B). A similar pattern was observed for MMP-9 (Fig. 2C). These parameters were also statistically significant in the multivariate analysis. However, levels of MMP-2 in noninflamed tissue and of MMP-2, MMP-9, TIMP-1, and TIMP-2 in inflamed tissue were not relevant to the risk for diagnostic or surgical recurrence (Table 3) and neither were the MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios (data not shown).

Discussion

In our relatively small cohort of 87 Dutch CD patients the T allele at SNP TIMP-1 372 T/C was found to increase the risk for surgical recurrence, although this effect just missed statistical significance in multivariate analysis. Patients carrying the T(T) or CT compared to C(C) genotype underwent re-resection much earlier (10.7 versus 19.1 years) after initial surgery. The TIMP-1 genotype had no effect on diagnostic recurrence, indicating a late onset or relatively mild influence of this polymorphism on CD progression. We also observed an increased risk on surgical (and diagnostic) recurrence in patients with a low TIMP-1 protein level in noninflamed intestinal tissue. Patients with TIMP-1 ≤ 2.88 ng/mg underwent re-resection after 10.7 years, almost 7 years earlier compared to patients with TIMP-1 levels above this value. We previously reported increased CD susceptibility and low TIMP-1 levels associated with the TIMP-1 T allele.[34] Combined with our current data, we postulate that this allele downregulates TIMP-1 expression by an as yet

Table 3. Cox proportional hazard analysis for testing MMP and TIMP protein levels in noninflamed and inflamed CD Tissue (*n* = 76 and 85, respectively) versus clinical parameters in relation to diagnostic (A) and surgical (B) recurrence after resection of diseased bowel.

A) Diagnostic Recurrence

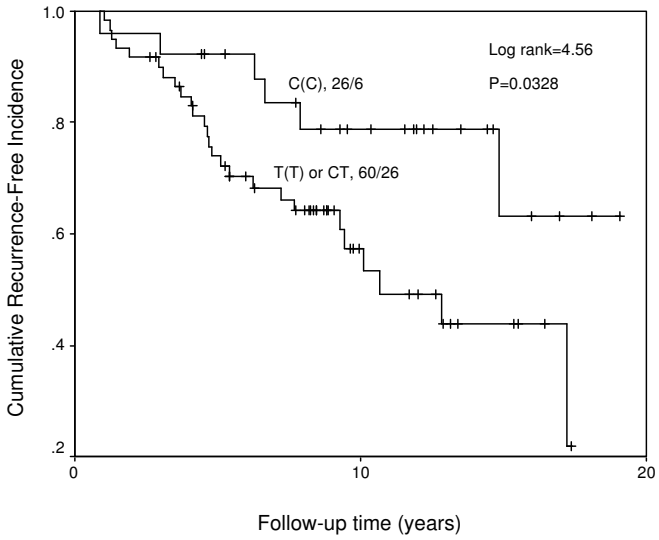
		Univariate			Multivariate		
Parameter	*N*	HR	95% CI	*P*	HR	95% CI	*P*
MMP-2 non-inflamed	38/		0.490-				
Median (60.23 ng/mg)	38	0.839	1.436	NS	1.024	0.529-1.982	NS
MMP-9 non-inflamed ≤ 5.11	16/		0.218-				
vs > 5.11 ng/mg	60	0.404	0.748	0.004	0.498	0.252-0.984	0.045
TIMP-1 non-inflamed ≤ 2.94	19/		0.287-				
vs > 2.94 ng/mg	57	0.509	0.902	0.021	0.658	0.350-1.237	NS
TIMP-2 non-inflamed ≤ 4.66	12/		0.263-				
vs > 4.66 ng/mg	64	0.513	1.003	0.051	0.661	0.313-1.396	NS
MMP-2 inflamed Median	43/		0.467-				
(110.85 ng/mg)	42	0.783	1.313	NS	0.701	0.387-1.269	NS
MMP-9 inflamed Median	43/		0.613-				
(37.63 ng/mg)	42	1.027	1.720	NS	1.134	0.664-1.938	NS
TIMP-1 inflamed Median	43/		0.550-				
(8.31 ng/mg)	42	0.923	1.548	NS	0.941	0.541-1.636	NS
TIMP-2 inflamed Median	43/		0.603-				
(6.9 ng/mg)	42	1.012	1.697	NS	1.385	0.789-2.432	NS

B) Surgical Recurrence

		Univariate			Multivariate		
Parameter	*N*	HR	95% CI	*P*	HR	95% CI	*P*
MMP-2 non-inflamed	38/		0.546-				
Median (60.23 ng/mg)	38	1.140	2.378	NS	1.226	0.483-3.116	NS
MMP-9 non-inflamed ≤ 5.11	16/		0.131-				
vs > 5.11 ng/mg	60	0.280	0.601	0.001	0.279	0.114-0.686	0.005
TIMP-1 non-inflamed ≤ 2.88	17/		0.200-				
vs > 2.88 ng/mg	59	0.434	0.942	0.035	0.403	0.174-0.932	0.034
TIMP-2 non-inflamed ≤ 4.66	12/		0.142-				
vs > 4.66 ng/mg	64	0.326	0.751	0.008	0.328	0.126-0.856	0.022
MMP-2 inflamed Median	43/		0.546-				
(110.85 ng/mg)	42	1.107	2.247	NS	0.979	0.450-2.130	NS
MMP-9 inflamed Median	43/		0.727-				
(37.63 ng/mg)	42	1.492	3.059	NS	1.772	0.845-3.716	NS
TIMP-1 inflamed Median	43/		0.535-				
(8.31 ng/mg)	42	1.084	2.198	NS	0.926	0.437-1.960	NS
TIMP-2 inflamed Median	43/		0.428-				
(6.9 ng/mg)	42	0.874	1.784	NS	0.998	0.471-2.114	NS

N, number of patients; HR, hazard ratio; CI, confidence interval; P, statistical significance

A Surgical recurrence TIMP-1 genotype



B Surgical recurrence TIMP-1 level

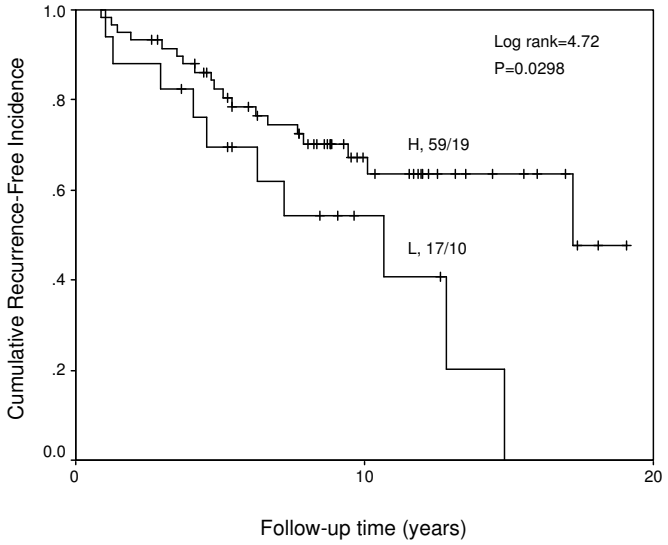


Figure 2. Kaplan–Meier analysis illustrating surgical recurrence-free incidence curves stratified by TIMP-1 SNP + 372 T/C (A), TIMP-1 level (B), and MMP-9 level (C, next page) in non-inflamed CD tissue

C Surgical recurrence MMP-9 level

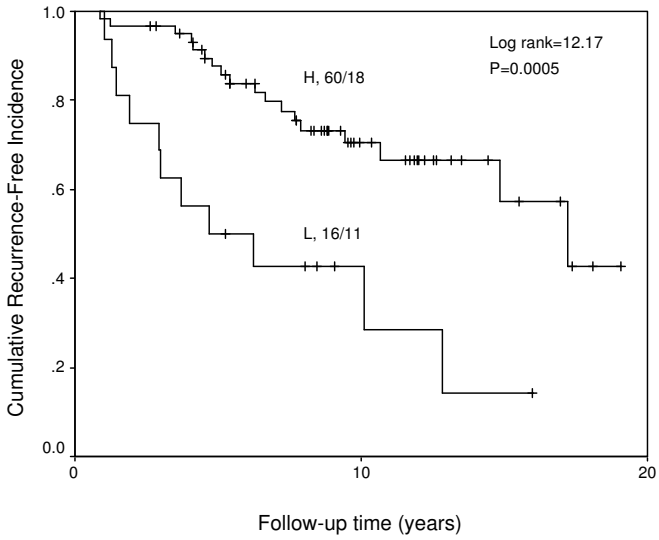


Figure 2, continued. Kaplan–Meier analysis illustrating surgical recurrence-free incidence curves stratified by TIMP-1 SNP + 372 T/C (A), TIMP-1 level (B), and MMP-9 level (C) in noninflamed CD tissue. Patients carrying the T(T) or CT compared to C(C) genotype underwent re-resection 10.7 versus 19.1 years after initial surgery ($P < 0.05$). High levels of both proteins were protective against surgical recurrence and increased median duration to re-resection from 10.7 (TIMP-1) or 4.7 (MMP-9) to 17.2 years in both, $P < 0.03$. H, L = high and low levels in noninflamed tissue (> and ≤ 2.88 [TIMP-1] versus > and ≤ 5.11 [MMP-9] ng/mg). X/Y = number of patients / number of patients with recurrence.

undefined mechanism, shifting the MMP/TIMP balance to a more proteolytic phenotype, enhancing CD susceptibility and worsening CD (postsurgical) prognosis. Alternatively, the TIMP-1 SNP is in linkage disequilibrium with another predisposing locus. The 372 SNP has been studied in other diseases with possible involvement of TIMP-1 in the pathogenesis, i.e., systemic sclerosis, intracranial and abdominal aneurysms, with inconclusive results.[43-45] CD patients with TIMP-2 levels >4.66 ng/mg in noninflamed tissue were also protected from developing surgical recurrence, presumably acting via a similar mechanism as described for TIMP-1. As TIMP-1/MMP-9 and TIMP-2/MMP-2 ratios were not associated with recurrence-free intervals, it appears that both TIMPs might exert their protective

effect through inhibition of other metalloproteinases, for instance MMP-3, -7, -12, or -13.[46-48] Remarkably, MMP-9 levels >5.11 ng/mg in non-inflamed tissue decreased the risk for diagnostic and surgical recurrence. In several models, targeting of MMP-9 attenuated chronic colitis, underlining the potential pathogenic role of this metalloproteinase in CD.[49,50] However, in CD several disease stages may be discerned and possibly, in the early acute phase, a small upregulation of MMP-9 in the macroscopically still normal tissue might be beneficial, whereas a large overexpression in the chronic active phase may lead to tissue ulceration and/or fibrosis. That would concur with our observation that the MMP and TIMP levels in the inflamed tissue are not prognostic to the diagnostic and surgical recurrence of CD. Recently, a protective role for MMP-2 (but not MMP-9) was demonstrated in experimental colitis, adding some credit to our hypothesis.[51] However, the exact mechanisms, proteolytic and nonproteolytic, by which the MMPs and TIMPs might be involved in the disease progression and recurrence is not completely clear. These factors are not only involved in tissue damage but also in intestinal wound healing, re-epitheliazation, myofibroblast and immune cell migration, scar formation, fibrogenesis, and neovascularization in the intestine, also after partial resection.[52-54] With regard to the clinical variables, we confirmed the effect of smoking also observed in previous publications.[15] Recently, cigarette smoke was shown to upregulate MMPs and induce a proinflammatory response.[55,56] We found, however, no relation between MMP and TIMP levels with smoking and the observed association of these proteins with recurrence was also independently significant in a multivariate model including smoking status. In summary, we have shown an association of the allele distribution at the TIMP-1 +372 T/C locus, the levels of TIMP-1, -2, and MMP-9 in non-inflamed tissue, and smoking habit with diagnostic and/or surgical recurrence of CD. Our results shed new light on the potential role of MMPs and TIMPs in the pathogenesis of recurrence/relapse in CD and may help in the identification of patients at risk, improving the effectiveness of current postoperative prophylactic treatment and disease management.[57,58]

Acknowledgments

The technical assistance of M. van den Berg, J.J. van der Reijden and W. van Duijn is greatly appreciated.

References

1. Kucharzik T, Maaser C, Lugering A, *et al*. Recent understanding of IBD pathogenesis: implications for future therapies. *Inflamm Bowel Dis.* 2006;12:1068–1083.
2. Gupta N, Cohen SA, Bostrom AG, *et al*. Risk factors for initial surgery in pediatric patients with Crohn's disease. *Gastroenterology.* 2006;130: 1069 – 1077.
3. Jess T, Riis L, Vind I, *et al*. Changes in clinical characteristics, course, and prognosis of inflammatory bowel disease during the last 5 decades: a population-based study from Copenhagen, Denmark. *Inflamm Bowel Dis.* 2007;13:481–489.
4. Rutgeerts P, Geboes K, Vantrappen G, *et al*. Predictability of the postoperative course of Crohn's disease. *Gastroenterology.* 1990;99: 956–963.
5. Wolters FL, Russel MG, Sijbrandij J, *et al*. Phenotype at diagnosis predicts recurrence rates in Crohn's disease. *Gut.* 2006;55:1124–1130.
6. Baldassano RN, Han PD, Jeshion WC, *et al*. Pediatric Crohn's disease: risk factors for postoperative recurrence. *Am J Gastroenterol.* 2001;96: 2169–2176.
7. Bergamaschi R, Pessaux P, Arnaud JP. Comparison of conventional and laparoscopic ileocolic resection for Crohn's disease. *Dis Colon Rectum.* 2003;46:1129–1133.
8. Bernell O, Lapidus A, Hellers G. Risk factors for surgery and postoperative recurrence in Crohn's disease. *Ann Surg.* 2000;231:38–45.
9. Ferrante M, de Hertogh G, Hlavaty T, *et al*. The value of myenteric plexitis to predict early postoperative Crohn's disease recurrence. *Gastroenterology.* 2006;130:1595–1606.

10. Hofer B, Bottger T, Hernandez-Richter T, *et al*. The impact of clinical types of disease manifestation on the risk of early postoperative recurrence in Crohn's disease. *Hepatogastroenterology.* 2001;48:152–155.
11. Meresse B, Rutgeerts P, Malchow H, *et al*. Low ileal interleukin 10 concentrations are predictive of endoscopic recurrence in patients with Crohn's disease. *Gut.* 2002;50:25–28.
12. Poggioli G, Laureti S, Selleri S, *et al*. Factors affecting recurrence in Crohn's disease. Results of a prospective audit. Int J Colorectal Dis.1996;11:294 –298.
13. Scarpa M, Angriman I, Barollo M, *et al*. Role of stapled and hand-sewn anastomoses in recurrence of Crohn's disease. *Hepatogastroenterology.* 2004;51:1053–1057.
14. Wagtmans MJ, Verspaget HW, Lamers CB, *et al*. Crohn's disease in the elderly: a comparison with young adults. *J Clin Gastroenterol.* 1998; 27:129 – 133.
15. Yamamoto T. Factors affecting recurrence after surgery for Crohn's disease. *World J Gastroenterol.* 2005;11:3971–3979.
16. Hanauer SB, Korelitz BI, Rutgeerts P, *et al*. Postoperative maintenance of Crohn's disease remission with 6-mercaptopurine, mesalamine, or placebo: a 2-year trial. *Gastroenterology*. 2004;127:723–729.
17. Rutgeerts P, Van Assche G, Vermeire S, *et al*. Ornidazole for prophylaxis of postoperative Crohn's disease recurrence: a randomized, double-blind, placebo-controlled trial. *Gastroenterology.* 2005;128:856 – 861.
18. Sorrentino D, Terrosu G, Avellini C, *et al*. Prevention of postoperative recurrence of Crohn's disease by infliximab. *Eur J Gastroenterol Hepatol.* 2006;18:457– 459.
19. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res.* 2006;69:562–573.
20. Baugh MD, Perry MJ, Hollander AP, *et al*. Matrix metalloproteinase levels are elevated in inflammatory bowel disease. *Gastroenterology.* 1999;117:814–822.
21. Bister VO, Salmela MT, Karjalainen-Lindsberg ML, *et al*. Differential expression of three matrix metalloproteinases, MMP-19, MMP-26, and MMP-

28, in normal and inflamed intestine and colon cancer. *Dig Dis Sci.* 2004;49:653– 661.

22. Heuschkel RB, MacDonald TT, Monteleone G, *et al*. Imbalance of stromelysin-1 and TIMP-1 in the mucosal lesions of children with inflammatory bowel disease. *Gut.* 2000;47:57– 62.
23. Lawrance IC, Fiocchi C, Chakravarti S. Ulcerative colitis and Crohn's disease: distinctive gene expression profiles and novel susceptibility candidate genes. *Hum Mol Genet.* 2001;10:445– 456.
24. Saarialho-Kere UK, Vaalamo M, Puolakkainen P, *et al*. Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. *Am J Pathol.* 1996;148:519 –526.
25. Stallmach A, Chan CC, Ecker KW, *et al*. Comparable expression of matrix metalloproteinases 1 and 2 in pouchitis and ulcerative colitis. *Gut.* 2000;47:415–422.
26. Vaalamo M, Karjalainen-Lindsberg ML, Puolakkainen P, *et al*. Distinct expression profiles of stromelysin-2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (MMP-12), and tissue inhibitor of metalloproteinases-3 (TIMP-3) in intestinal ulcerations. *Am J Pathol.* 1998;152:1005–1014.
27. von Lampe B, Barthel B, Coupland SE, *et al*. Differential expression of matrix metalloproteinases and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease. *Gut.* 2000;47:63–73.
28. Gao Q, Meijer MJ, Kubben FJ, *et al*. Expression of matrix metalloproteinases-2 and -9 in intestinal tissue of patients with inflammatory bowel diseases. *Dig Liver Dis.* 2005;37:584 –592.
29. Meijer MJ, Mieremet-Ooms MA, van der Zon AM, *et al*. Increased mucosal matrix metalloproteinase-1, -2, -3 and -9 activity in patients with inflammatory bowel disease and the relation with Crohn's disease phenotype. *Dig Liver Dis.* 2007;39:733–739.
30. Warnaar N, Hofker HS, Maathuis MH, *et al*. Matrix metalloproteinases as profibrotic factors in terminal ileum in Crohn's disease. *Inflamm Bowel Dis.* 2006;12:863– 869.

31. Komatsu M, Kobayashi D, Saito K, *et al*. Tumor necrosis factor-alpha in serum of patients with inflammatory bowel disease as measured by a highly sensitive immuno-PCR. *Clin Chem.* 2001;47:1297–1301.
32. Pender SL, Fell JM, Chamow SM, *et al*. A p55 TNF receptor immuno-adhesin prevents T cell-mediated intestinal injury by inhibiting matrix metalloproteinase production. *J Immunol.* 1998;160:4098 – 4103.
33. Rutter JL, Mitchell TI, Buttice G, *et al*. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res.* 1998;58:5321–5325.
34. Meijer MJ, Mieremet-Ooms MA, van Hogezand RA, *et al*. Role of matrix metalloproteinase, tissue inhibitor of metalloproteinase and tumor necrosis factor-alpha single nucleotide gene polymorphisms in inflammatory bowel disease. *World J Gastroenterol.* 2007;13:2960–2966.
35. Pender SL, Croucher PJ, Mascheretti S, *et al*. Transmission disequilibrium test of stromelysin-1 gene variation in relation to Crohn's disease. *J Med Genet.* 2004;41:e112.
36. Cantor MJ, Nickerson P, Bernstein CN. The role of cytokine gene polymorphisms in determining disease susceptibility and phenotype in inflammatory bowel disease. *Am J Gastroenterol.* 2005;100:1134 –1142.
37. Ferreira AC, Almeida S, Tavares M, *et al*. NOD2/CARD15 and TNFA, but not IL1B and IL1RN, are associated with Crohn's disease. *Inflamm Bowel Dis.* 2005;11:331–339.
38. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988; 16:1215.
39. Kubben FJ, Sier CF, Meijer MJ, *et al*. Clinical impact of MMP and TIMP gene polymorphisms in gastric cancer. *Br J Cancer.* 2006;95: 744 –751.
40. Meijer MJ, Mieremet-Ooms MA, van Duijn W, *et al*. Effect of the anti-tumor necrosis factor-alpha antibody infliximab on the ex vivo mucosal matrix metalloproteinase-proteolytic phenotype in inflammatory bowel disease. *Inflamm Bowel Dis.* 2007;13:200 –210.
41. Kuyvenhoven JP, van Hoek B, Blom E, *et al*. Assessment of the clinical

significance of serum matrix metalloproteinases MMP-2 and MMP-9 in patients with various chronic liver diseases and hepatocellular carcinoma. *Thromb Haemost.* 2003;89:718 –725.

42. Lowry OH, Rosebrough NJ, Farr AL, *et al*. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193:265–275.
43. Hinterseher I, Krex D, Kuhlisch E, *et al*. Tissue inhibitor of metalloproteinase-1 (TIMP-1) polymorphisms in a Caucasian population with abdominal aortic aneurysm. *World J Surg.* 2007;31:2248 –2254.
44. Indelicato M, Chiarenza V, Libra M, *et al*. Analysis of TIMP-1 gene polymorphisms in Italian sclerodermic patients. *J Clin Lab Anal.* 2006; 20:173–176.
45. Krex D, Rohl H, Konig IR, *et al*. Tissue inhibitor of metalloproteinases-1, -2, and -3 polymorphisms in a white population with intracranial aneurysms. *Stroke.* 2003;34:2817–2821.
46. Gordon JN, Pickard KM, Di Sabatino A, *et al*. Matrix metalloproteinase-3 production by gut IgG plasma cells in chronic inflammatory bowel disease. *Inflamm Bowel Dis.* 2008;14:195–214.
47. Pender SL, Li CK, Sabatino AD, *et al*. Role of macrophage metalloelastase in gut inflammation. *Ann N Y Acad Sci.* 2006;1072:386 –388.
48. Rath T, Roderfeld M, Graf J, *et al*. Enhanced expression of MMP-7 and MMP-13 in inflammatory bowel disease: a precancerous potential? *Inflamm Bowel Dis.* 2006;12:1025–1035.
49. Castaneda FE, Walia B, Vijay-Kumar M, *et al*. Targeted deletion of metalloproteinase 9 attenuates experimental colitis in mice: central role of epithelial-derived MMP. *Gastroenterology.* 2005;129:1991–2008.
50. Ishida K, Takai S, Murano M, *et al*. Role of chymase-dependent matrix metalloproteinase-9 activation in mice with dextran sodium sulfate-induced colitis. *J Pharmacol Exp Ther.* 2008;324:422– 426.
51. Garg P, Rojas M, Ravi A, *et al*. Selective ablation of matrix metalloproteinase-2 exacerbates experimental colitis: contrasting role of gelatinases in the pathogenesis of colitis. *J Immunol.* 2006;177:4103– 4112.
52. Medina C, Radomski MW. Role of matrix metalloproteinases in intestinal

inflammation. *J Pharmacol Exp Ther.* 2006;318:933–938.

53. Naito Y, Yoshikawa T. Role of matrix metalloproteinases in inflammatory bowel disease. *Mol Aspects Med.* 2005;26:379 –390.
54. Ravi A, Garg P, Sitaraman SV. Matrix metalloproteinases in inflammatory bowel disease: boon or a bane? *Inflamm Bowel Dis.* 2007;13:97–107.
55. Katono T, Kawato T, Tanabe N, *et al*. Nicotine treatment induces expression of matrix metalloproteinases in human osteoblastic Saos-2 cells. *Acta Biochim Biophys Sin (Shanghai).* 2006;38:874 – 882.
56. Nordskog BK, Blixt AD, Morgan WT, *et al*. Matrix-degrading and pro-inflammatory changes in human vascular endothelial cells exposed to cigarette smoke condensate. *Cardiovasc Toxicol.* 2003;3:101–117.
57. Lemann M. Review article: can post-operative recurrence in Crohn's disease be prevented? *Aliment Pharmacol Ther.* 2006;24(Suppl 3):22–28.
58. Sandborn WJ, Feagan BG. The efficacy of azathioprine and 6-mercaptopurine for the prevention of postoperative recurrence in patients with Crohn's disease remains uncertain. *Gastroenterology.* 2004;127:990–993.