Liver transplantation : chimerism, complications and matrix metalloproteinases

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Chapter 6

Matrix metalloproteinase 2 genotype is associated with nonanastomotic biliary strictures after orthotopic liver transplantation

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Abstract

Background

Nonanastomotic biliary strictures (NAS) are a serious complica-tion after orthotopic liver transplantation (OLT). Matrix metalloproteinases (MMPs) are involved in connective tissue remodelling in chronic liver disease and complications after OLT.

Aim

To evaluate the relationship between MMP-2 and MMP-9 gene polymorphisms and NAS.

Methods

MMP-2 (- 1306 C/T) and MMP-9 (- 1562 C/T) gene promoter polymorphisms were analysed in 314 recipient-donor combinations. Serum levels of these MMPs were determined in subgroups of patients as well. NAS were identified with various radiological imaging studies performed within 4 years after OLT and defined as any stricture, dilation or irregularity of the intra- or extrahepatic bile ducts of the liver graft followed by an intervention, after exclusion of hepatic artery thrombosis and anastomotic strictures.

Results

The average incidence of NAS was 15%. The major clinical risk factor for the development of NAS was PSC in the recipient. The presence of the MMP-2 CT genotype in donor and/or recipient was associated with a significantly higher incidence of NAS, up to 29% when both donor and recipient had the MMP-2 CT genotype (P = 0.003). In the multivariate analyses, pre-OLT PSC (hazard ratio 2.1, *P* = 0.02) and MMP-2 CT genotype (hazard ratio 3.5, *P* = 0.003) were found to be independent risk factors for the development of NAS after OLT. No obvious association was found between NAS and the MMP-9 genotype and serum levels of the MMPs.

Conclusion

MMP-2 CT genotype of donor and recipient is an independent risk factor, in addition to PSC, for the development of NAS after OLT.

Biliary complications are a common feature after orthotopic liver transplantation (OLT), with a reported in-cidence of up to 35%. Leaks and strictures are the most common complications, often requiring endoscopic, radiological or surgical intervention[1-9]. Anastomotic strictures result from surgical or local ischaemic causes. Main categories of risk factors for nonanastomotic biliary strictures (NAS) include ischaemia-related injury, immunologically induced injury and cytotoxic injury by bile salts. A higher incidence of NAS is reported in patients transplanted for primary sclerosing cholangitis (PSC) and patients who suffered from a postoperative CMV infection[10-17]. Donation after cardiac death (DCD) procedures are also reported to have an increased risk of NAS compared with donation after brain death procedures[18,19]. NAS are often referred to as ischae-mic-type biliary lesions, based on the resemblance with biliary abnormalities observed after hepatic artery thrombosis. The reported incidence of NAS varies in different publications from 1 to 19%[4,5,20-25]. If untreated, NAS may lead to cholestasis, severe graft dysfunction, septic complications, secondary cirrhosis and graft loss[7,23,26]. Matrix metalloproteinases (MMPs) comprise a large family of proteolytic enzymes that are important in physiological and disease-related extracellular matrix remodelling processes[27-30]. MMP-2 and MMP-9 are capable of digesting components of the connective tissue matrix and type IV collagen within basement mem-branes. These MMPs are considered to play an important role in cancer development, tissue remodelling, fibrosis and inflammation, including cirrhosis and liver transplantation[31-35]. We showed previously, for example, that serum MMP-2 levels increased, whereas MMP-9 levels decreased in relation to the severity of the cirrhosis[31]. These serum MMP levels were subsequently found to change irrespective of their gene polymorphisms in late phase injury or rejection (I/R) after liver transplantation[36]. The aim of the present study was to assess whether a relationship exists between MMP-2 and MMP-9 gene promoter polymorphisms in the donor and recipient DNAwith the development of NAS after OLT.

Patients and methods

Patients

All adult patients who received a liver transplant at the Leiden University Medical Center (LUMC) and University Medical Center Groningen (UMCG) in the Netherlands were eligible for inclusion. For this study, 202 patients were identified from the transplantation databases who underwent OLT at the LUMC between 1992 and 2005, of whom

we were able to include 147 patients whose DNAwas available from both donor and recipient, and who had at least 7 days of follow-up after liver transplantation. Also, patients who received OLT be-tween 2000 and 2005 at the UMCG were eligible for the study because data were available. Of the 224 available patients, 167 unselected patients could be included of whom we had DNA from both recipient and donor, and who had at least 7 days of follow-up after transplanta-tion. Genomic DNA was extracted routinely from per-ipheral blood and/or tissue samples without given preference to any explicit clinical variables. All patients received standard immunosuppressive therapy consisting ofcorticosteroids, a calcineurin inhibitor (i.e., cyclospor-ine or tacrolimus) with or without mycophenolate mofetil or azathioprine and/or basiliximab. Azathioprine was used until 2001, and thereafter mycophenolate mofetil was given in case of impaired renal function. Demographical and clinicopathological characteristics of the recipient at the time of OLT (age, gender, indication for liver transplantation, laboratory MELD score), donor information (age, gender and donor type), transplanta-tion procedure variables (warm and cold ischaemia time) and post-transplant follow-up data of up to 4 years were collected from the transplantation databases.

This study was performed with informed consent from the patients according to the guidelines of the Medical Ethics Committee of both participating centres and in compliance with the Helsinki Declaration.

Nonanastomotic strictures

In this study, only biliary strictures followed that by an intervention were included. If a biliary stricture was suspected from clinical findings, liver function tests or abdominal ultrasound, further imaging of the biliary tract was performed. In both centres, a biliary drain was placed routinely after OLT and cholangiography was performed if clinically indicated and in the LUMC, cholangiography was also performed routinely 6 weeks after OLT. All imaging studies of the biliary tree, performed within 4 years after OLT, were included [direct cholangiography via the biliary drain, percuta-neous transhepatic cholangiodrainage (PTCD), ERCP as well as MRCP]. For the purpose of this study, NAS were defined as follows: any stricture, dilation or irregularity of the intrahepatic or extrahepatic bile ducts of the liver graft, either with or without biliary sludge formation, at least 1 cm above the biliary anastomosis and treated endoscopically with ERCP and dilation and/or stenting, percutaneously with PTCD or by surgical intervention. Hepatic artery thrombosis by either Doppler ultrasound or conventional angiography

as well as isolated strictures/ stenoses at the bile duct anastomosis and related dila-tions were, by definition, excluded from this analysis.

Genotyping
Genomic DNA was extracted by routine methods from peripheral blood leucocytes and/or tissue samples. In addition, DNA samples from the blood or tissue of the liver donor were obtained from the Eurotransplant Reference Laboratory or freshly isolated.

MMP-2: high-resolution DNA melting analysis
MMP2 1306 C/T (rs243865) genotyping, as most relevant SNP, was performed with the use of high-resolution DNA melting assay[37]. Sequences of the polymerase chain reaction (PCR) primers were 5′-CCAGTGCCTC TTGCTGTTTT-3′ (forward) and 5′- GACTTCTGAGC TGAGACCTGA-3′ (reverse). The unlabelled probe was designed according to the wild-type (C) genotype and had the following sequence: 5′-CCACCCAGCACTCCACCTCTTTAGCTC-3′. The probe had a 3′-amino-C7 modification to prevent DNA polymerase extension during PCR. In brief, high-resolution melting analysis of PCR products amplified in the presence of a satu-rating double-stranded DNA dye (LCGreenPlus, Idaho Technology, Salt Lake City, Utah, USA) and a 3′-blocked probe, identified both heterozygous and homozygous sequence variants. Heterozygotes were identified by a change in melting curve shape, and different homozy-gotes are distinguished by a change in melting tempera-ture. In each experiment, sequence-verified control donors for each genotype were used.

MMP-9: PCR-RFLP genotyping
The MMP-9 SNP C/T at position - 1562 (rs3918242) was determined with PCR analysis followed by restric-tion enzyme fragment length polymorphisms (RFLP) analysis, the principles of which are described elsewhere[36], and confirmed by direct sequence analysis of four patients. Briefly, the region flanking the SNP was amplified with outer primers 5′-ATGGCTCATGCCCGTAATC-3′ and 5′-TCACCTTCTTCAAAGCCCTATT-3′ followed by RFLP analysis with *Sph*I to produce 352, 35212071145 or 2071145 bp fragments in case of CC, CT and TT genotype respectively. Genotypes CC, CT and TT are easily identified from the migration pattern on agarose gels[36,38-40].

Determination of serological MMP levels
From two subgroups of patients included in our study, we also

assessed the serological levels of MMP-2 and MMP-9 before and after transplantation. This pretrans-plantation group consisted of 47 patients (30 males) with chronic liver disease of various aetiologies, including 27 patients who eventually underwent an OLT. Their med-ian age was 46 years (range 16-68). Fourteen patients had chronic viral hepatitis, 14 patients had cholestatic liver disease, 10 patients had alcohol-related liver disease and the remaining nine patients had miscellaneous liver diseases. From the group of 27 OLT patients, serum samples 1 month after transplantation were evaluated. All serum samples had been stored at - 80 °C until use. MMP-2 and MMP-9 concentrations were determined using highly specific enzyme-linked immunosorbent assays, which measures the pro-enzyme, active- and inhibitor complexed forms, as described previously[31,36].

Statistical analysis

Data were analysed using spss 17.0 software (SPSS Inc.; Chicago, IL, USA). Characteristics of the liver transplant recipients, donors and post-transplant follow-up data with the risk of developing NAS were analysed using the log-rank and two-tailed Student's *t*-tests. Differences in the serological levels of MMP were analysed using ANOVA.

Genotype frequencies were analysed by generating two-by-three contingency tables and statistical analysis was performed using the X^2-test or the Fisher's exact test, where appropriate. Comparison of time with NAS was made using Kaplan-Meier statistics with a log-rank test. Univariate and multivariate analyses were performed using Cox's proportional hazards method. Variables associated with an increased risk of NAS at the $P \leq 0.15$ level in the univariate logistic regression analysis were included in the backward stepwise multivariate logistic regression model. *P*-values ≤ 0.05 were considered statistically significant.

Results

The study population consisted of 314 OLT donor/ recipient combinations of which 48 (15%) developed NAS within the first 4 years after transplantation.

MMP-2 genotype and NAS

The frequencies of MMP-2 and MMP-9 gene promoter polymorphisms in recipients and in donors vs the occurrence of NAS are given in Table 1. Evaluation whether the MMP genotype is reflected in the serum level indicated that in patients with liver disease no such relation exists.

Table 1. Frequencies of matrix metalloproteinase polymorphisms in orthotopic liver transplant recipients and donors (*n* = 314)

Genotype				Recipient		Donor	
dbSNP ID	SNP			NAS %(*n*)	NoNAS %(*n*)	NAS %(*n*)	No NAS %(*n*)
MMP-2	— 1306	C → T	CC	46 (22)	61 (162)	44 (21)	53 (142)
rs243865			CT	52 (25)	33 (87)	56 (27)	41 (108)
			TT	2 (1)	6 (17)	0 (0)	6 (16)
				$P < 0.03$, X^2 7.2		$P = 0.05$, X^2 5.9	
MMP-9	— 1562	C → T	CC	67 (32)	71 (185)	75 (36)	78 (206)
rs3918242			CT	33 (16)	27 (71)	23 (11)	21 (56)
			TT	0 (0)	2 (5)	2 (1)	1 (2)
				$P = 0.46$, X^2 1.6(n = 309)		$P = 0.66$, X^2 0.9(n = 312)	

Table 2. Comparison of donor, recipient and procedure variables between patients with and without nonanastomotic biliary strictures after orthotopic liver transplant (*n* = 314)

	Total	NAS (*n* = 48)	%	No NAS (*n* = 266)	%	*P*-value
Donor variables						
Age (in years, median, range)	44 (16–72)	43	(16–67)	44	(9–72)	0.80
Gender						
Female	153	29	60	124	47	0.08
Male	161	19	40	142	53	
Recipient variables						
Age (in years, median, range)	48 (16–70)	45	(16–61)	48	(17–70)	0.08
Gender						
Female	122	16	33	106	40	0.39
Male	192	32	67	160	60	
Primary liver disease						**0.04**
Post viral cirrhosis	59	4	8	55	21	
Alcoholic cirrhosis	46	5	10	41	15	
PSC	57	15	31	42	16	
Other cholestatic disease*	28	6	13	22	8	
Other disease†	124	18	38	106	40	
Laboratory MELD score (median, range)	15	15 (6–40)		15 (6–40)		0.98
OLT procedure variables						0.50
DCD	25	5	10	20	8	
DBD	289	43	90	246	92	
WIT in minutes (mean±SD)	44±13 *n* = 296	42±10 *n* = 46		44±13 *n* = 250		0.37
CIT in minutes (mean±SD)	573±188 *n* = 299	595±183 *n* = 46		561±189 *n* = 253		0.38
MMP-2 [rs243865] CT						**0.003**
No CT present	115	10	21	105	39	
CT in recipient or donor	151	24	50	127	48	
CT in recipient and donor	48	14	29	34	13	

Age, MELD scores, WIT and CIT differences were evaluated by Student's t-test; frequency distribution data were analysed by w2 or Fisher's exact tests, where appropriate.

CIT, cold ischaemia time; time between the start of cold perfusion of graft in the donor and the end of cold preservation of the liver graft; DBD, donation after brain death; DCD, donation after cardiac death; MELD, model for end-stage liver disease; MMP, matrix metalloproteinase; NAS, nonanastomotic biliary lesions; OLT, orthotopic liver transplantation; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SD, standard deviation; SSC, secondary sclerosing cholangitis; WIT, warm ischaemia time; time between the end of cold ischaemic preservation of the liver graft and portal vein reperfusion in the recipient.

*Other cholestatic disease comprise PBC and SSC.

†Other diseases include predominantly autoimmune hepatitis, cryptogenic cirrhosis and metabolic disorders.

Specifically, MMP-2 levels in the pre-OLT serum of recipients with a CC (n = 32) genotype was 5123 ± 553ng/ml, whereas for those with a CT or TT genotype (n = 15), these levels were 5347 ± 886 (NS). For MMP-9, these levels were 129 ± 16 (n = 36) vs 156 ± 28 (n = 11) respectively (NS). The presence of MMP-2 CT genotype in the recipient as well as in the donor was significantly associated with the development of NAS. Furthermore, the cumulative presence of MMP-2 CT genotype in both recipient and donor vs the occurrence ofNAS is shown in Tables 2 and 3. In the group of patients that developed NAS, the absence of a CT genotype was more frequent (21%) than in the patients that did not develop NAS (39%) and for CT in donor and recipient, exactly the opposite was observed (29% vs 13%, Table 2). If CT genotype was present in neither recipient nor donor, the risk of developing NAS was 9% (10/115). When MMP-2 CT genotype was present in either donor or recipient, NAS developed in 16% (24/151) of cases. The occurrence of NAS increased to 29% if MMP-2 CT genotype was present in both recipient and donor (14/48; $P < 0.003$, Table 3). Figure 1 shows the cumulative incidence ofNAS within 48 months after OLT related to the presence of MMP-2 CT genotype in recipient and donor. We also evaluated whether this association between genotype MMP-2 and NAS was reflected in the serum levels. One month after OLT, the MMP-2 level in patients with NAS was showed a trend to be lower [i.e., 1892 ± 431ng/ml (n = 5) vs 2869 ± 287 (n = 22), P = 0.06], compared with the patients without NAS. Interestingly, a similar trend was observed in relation to the MMP-2 genotype, i.e. lower in relation to the presence of CT [2969 ± 452 vs 2540 ± 349 vs 2396 ± 448 for no CT in donor or recipient (n = 10), CT in donor or recipient (n = 15) and CT in donor and recipient (n = 2), respectively, NS].

Further assessment of the impact of the MMP-2 genotypes and NAS-related morbidity by including re-OLTs showed a similar stepwise increase in relation to the MMP-2 genotype from 14% (16/115) to 20% (30/151) and 38% (18/48) respectively (X^2 11.66, P = 0.003). By including death in the follow-up, this increased to 26% (30/115), 29% (44/151) and 44% (21/48) respectively (X^2 5.18, P = 0.08).

In a similar manner, the MMP-9 genotype distribution of recipient and donor vs the occurrence of NAS was evaluated. However, no significant correlation was found between MMP-9 genotype and the development of NAS (Table 1) or with the serum levels of MMP-9 (data not shown).

Multivariate analysis of MMP-2 genotype and covariates

The development of NAS was significantly higher when PSC was the

Table 3. Univariate and multivariate analysis for the association of risk factors of nonanastomotic biliary lesions in orthotopic liver transplant patients

				Univariate analysis		Multivariate analysis (n = 299)	
Risk factor		**NAS**	**%**	**HR (95% CI)**	***P*-value**	**Adjusted HR (95% CI)**	***P*-value**
MMP-2 [rs243865] CT					0.01		0.008
Donor and recipient	Vs. none	14/48	29	3.48 (1.55–7.84)	0.003	3.48 (1.54–7.87)	0.003
Donor or recipient	Vs. none	24/151	16	1.84 (0.88–3.84)	0.11	1.64 (0.78–3.46)	0.20
None	Reference	10/115	9	1 (reference)		1 (reference)	
Age recipient	Continuous	48/314		0.98 (0.96–1.01)	0.13		
Age donor	Continuous	48/314		1.00 (0.98–1.02)	0.72		
Gender recipient	Female	16/122	13	0.74 (0.41–1.35)	0.32		
	Male	32/192	17	1 (reference)			
Gender donor	Female	29/153	19	1.53 (0.86–2.73)	0.15		
	Male	19/161	12	1 (reference)			
Primary liver disease	PSC	15/57	26	2.0 (1.11–3.76)	0.02	2.14 (1.13–4.06)	0.02
	Other	33/257	13	1 (reference)			
Laboratory MELD score	Continuous	48/314		1.00 (0.97–1.04)	0.84		
Procedure	DCD	5/25	20	1.50 (0.60–3.80)	0.39		
	DBD	43/289	15	1 (reference)			
CIT	Continuous	46/299		1.000 (1.000–1.002)	0.15	1.001 (1.000–1.003)	0.08
WIT	Continuous	46/296		0.99 (0.96–1.01)	0.38		

CI, confidence interval; CIT, cold ischaemia time; time between the start of cold perfusion of graft in the donor and the end of cold preservation of the liver graft; DBD, donation after brain death; DCD, donation after cardiac death; HR, hazard ratio; MELD, model for end-stage liver disease; MMP, matrix metalloproteinase; NAS, nonanastomotic biliary strictures; OLT, orthotopic liver transplantation; PSC, primary sclerosing cholangitis; WIT, warm ischaemia time; time between the end of cold ischaemic preservation of the liver graft and portal vein reperfusion in the recipient.
Univariate and backward multivariate analyses were performed using Cox's proportional hazards method.

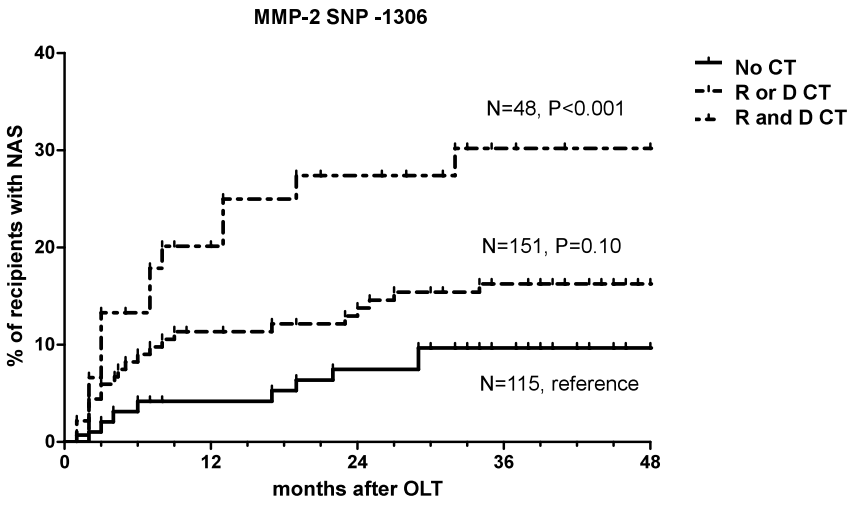

Fig. 1.
Cumulative incidence of NAS within 48 months after OLT related to the presence of MMP-2 CT genotype in recipient (R) and donor (D). MMP, matrix metalloproteinase; NAS, nonanastomotic biliary strictures; OLT, orthotopic liver transplantation.

indication for OLT [15/48 cases (31%) vs 33/ 257 cases (13%)], as expected. No significant association was found between the occurrence of NAS and other transplant characteristics, such as gender and age (both of recipient and donor), laboratory MELD score, length of warm or cold ischaemia time or DCD procedures. However, it should be noted that only 25 DCD procedures were included in this cohort. Also, in relation to immunosuppressive therapy, there was no association found with the development of NAS, i.e. patients on corticosteroids with a calcineurin inhibitor with or without basiliximab had a similar risk of developing NAS [13% (27/209) vs 20% (21/105), respectively, NS].

Early ($\leq$ 12 months) and late (12-48 months) onset of NAS was also looked at for all studied risk factors. NAS was diagnosed in 33 cases (10.5%) within the first year after OLT and in 15 cases (4.8%) from 12 to 48 months after OLT. Cold ischaemia time (CIT) was significantly longer for the group with early development of NAS (631 ± 179 vs 520± 174min; P =0.05). Interestingly, the effect of increased CIT and the incidence of NAS was particularly present in the first 12 months after OLT (hazard ratio 1.002; P = 0.03). Late occurrence of NAS was observed relatively more frequently when patients were transplanted for PSC. With PSC as the indication for liver transplantation, the occurrence of NAS within the first 12 months after OLT was 27% (9/33) as opposed to 40% (6/15) from the late onset NAS. A pre-OLT diagnosis of PSC was found to be accompanied particu-larly with an increased risk of late onset NAS (hazard ratio 3.1; P = 0.03).

Multivariate Cox regression analyses and the backward elimination procedure, taking all patient and transplant characteristics into account with an increased risk ofNAS (at the level of$P \leq 0.15$), indicated that the presence of MMP-2 CT genotype in donor and recipient was an independent risk factor for the development ofNAS with a higher hazard ratio than PSC as primary liver disease (3.5 vs 2.1, respectively; Table 3).

Discussion

In the present study, we report a strong association between the presence of MMP-2 CT genotype in donor and/or recipient and the development of NAS after OLT. In fact, MMP-2 genotype was a greater risk factor for NAS after OLT than PSC. The presence ofthe MMP-2 CT genotype in donor and/or recipient was found to increase the NAS incidence stepwise from 9% when absent, increasing to 16% when present in either recipient or donor, further increasing to 29% when present in both donor and recipient. In contrast, no association was

found between MMP-9 genotype and the development of NAS. Nonanastomotic strictures are considered to be the most troublesome biliary complication after OLT, associated with high retransplant rates in up to 20% of patients[5,7,25,41]. Interestingly, further assessment of the impact of the MMP-2 genotypes and NAS-related morbidity in our patients, by including re-OLTs, also revealed a stepwise increase in relation to the MMP-2 genotype from 14 to 38% and by including death in the follow-up, this increased even up from 26 to 44%. Apparently, the MMP-2 CT genotype also contributes to the morbidity accompanying NAS in the OLT patients.

Various risk factors for NAS have been identified, suggesting a multifactorial origin [9,14]. The main categories include ischaemia-related injury, immune-mediated injury such as ABO compatibility, pre-existing disease (especially PSC) and toxic injury by bile salts[42,43]. In addition to clinicopathological factors, we were also interested in the impact of the gelatinases MMP-2 and MMP-9 in the development of NAS.

Matrix metalloproteinases comprise a large family of proteolytic enzymes involved in physiological and dis-ease-related connective tissue remodelling processes and the gelatinases MMP-2 and MMP-9 are considered to play an important role in inflammation, degradation and remodelling processes in the liver[29,31,44]. MMP activity is regulated by various factors and controlled by activation of latent pro-enzymes and by interaction with endogenous inhibitors such as tissue inhibitors of metal-loproteinases (TIMPs). Recently, several single nucleotide polymorphisms (SNP) in the gene promoter regions of MMPs have been found with an impact on the transcription rate (30, 32). The C/T transition at posi-tion — 1306 in the promoter ofMMP-2, which abolishes the Sp 1 binding site, and leads to decreased mRNA transcription and protein expression, is generally ac-cepted to be the most relevant SNP for MMP-2. Other SNPs of MMP-2 have been reported as well, e.g. — 1575 G/A, — 790 C/T and — 735 C/T, but these are in almost complete linkage (dis)equilibrium with — 1306 C/Tand thus provide no additional information[30,32,33]. In several studies, an association was demonstrated between MMP-2 polymorphisms and the development of cancer. It has even been suggested that MMP-2 represents a potential target for tumour therapeutics[32,33].

In the MMP-9 gene, an SNP at position — 1562 is because of a C to T substitution in the promoter region. *In vitro* studies have shown that this transition results in loss of binding of a nuclear repression protein and increased transcriptional activity in macrophages, associated with the severity of coronary atherosclerosis.

Although other SNPs in the MMP-9 gene have been described, they were mainly nonsynonomous located in the exon part of the gene and found not to affect the activity or level of the enzyme[34,35] In contrast, in cardiovascular disease, for example, the — 1562 T allele was found to be associated with increased MMP-9 plasma levels[35].

In the liver, the hepatic stellate cell seems to be the main cellular source ofMMP-2 and when activated these cells are involved in the synthesis of matrix proteins and in the regulation of matrix degradation leading to liver fibrosis. Following liver injury, the stellate cells become activated and can express a wide range of MMPs and TIMPs, but in particular MMP-2[44-46]. Increased mRNA expression of MMP-2 was reported in liver biopsies of patients with cirrhosis[44]. We found pre-viously serum levels ofMMP-2 to be increased in patients with chronic liver disease and strongly correlated with serum markers indicative of a poor liver function[31]. After OLT, a gradual decrease of MMP-2 levels were found they remained higher, however, than found in healthy controls and increased with recurrent liver dis-ease. MMP-9 is released predominantly from neutrophils and macrophages, but the principal source in the liver is thought to be the Kupffer cell, the resident macrophage of the liver. Taking into account the different hepatic sources of MMP-2 (parenchymal stellate cells) and MMP-9 (Kupffer cells), one would expect a more long-standing effect of MMP-2 polymorphisms compared with MMP-9 polymorphisms. This is in line with our findings on the development of NAS within 4 years after OLT.

MMP-2 and MMP-9 seem to play a critical role in cold storage preservation injury and the reperfusion injury of liver grafts. We found previously elevated serum levels of MMP-9 at 1 week after OLT in patients with acute allograft rejection[45], illustrating that the extracellular matrix might be an important target in the process of acute rejection. In a recent study, however, we found serum levels to be affected but not in association with the MMP-2 and MMP-9 polymorphisms in late phase ischaemia/reperfusion I/R after OLT[36]. Also in the present study, we did not find that the genotype of the MMPs was reflected in the respective serum level of the patients before OLT. After transplantation, however, the MMP-2 levels showed a trend to be lower in patients that developed NAS and in association with the — 1306 CT genotype of recipient and donor. These observations might indicate a genetically determined reduced MMP-2 tissue remodelling as a cause of NAS after OLT, but the number of patients evaluated is too low to draw definite conclusions. With respect to MMP-9, no genotype (— 1562 CT)-phenotype (serum level) association was found in relation to NAS.

In the present study, early development of NAS (≤ 12 months) was associated with longer cold ischaemia times. It has been well described in previous studies that patients who are transplanted for PSC have a higher incidence of NAS after transplantation[7,20,21,47,48]. Our results confirm this finding and the multivariate analysis shows PSC to be a risk factor for the develop-ment of NAS, independent of MMP-2 genotype and occurring late after OLT (Table 3). The multivariate analysis shows MMP-2 genotype in donor and recipient to be a much stronger predictor for the development of NAS (hazard ratio 3.5) than a pre-OLT diagnosis of PSC (hazard ratio 2.1).

In summary, MMP-2 CT genotype in both donor and recipient is strongly and independently related to the development of NAS within 4 years after OLT. No association was found with MMP-9 CT genotype. These observations merit further studies on the influence of donor and recipient MMP-2 genotypes and on the MMP-related mechanisms involved in the development of NAS after OLT. As NAS leads to significant morbidity and graft loss in OLT, MMP-2 gene-based identification of these high-risk patients might be of great clinical importance.

References

1. Park JS, Kim MH, Lee SK, *et al.* Efficacy of endoscopic and percutaneous treatments for biliary complications after cadaveric and living donor liver transplantation. *Gastrointest Endosc* 2003; **57:** 78-85.

2. Qian YB, Liu CL, Lo CM, Fan ST. Risk factors for biliary complications after liver transplantation. *Arch Surg* 2004; **139:** 1101-5.

3. Rerknimitr R, Sherman S, Fogel EL, *et al.* Biliary tract complications after orthotopic liver transplantation with choledochocholedochostomy anastomosis: endoscopic findings and results of therapy. *Gastrointest Endosc* 2002; **55:** 224-31.

4. Sawyer RG, Punch JD. Incidence and management of biliary complications after 291 liver transplants following the introduction of transcystic stenting. *Transplantation* 1998; **66:** 1201-7.

5. Thethy S, Thomson BN, Pleass H, *et al.* Management of biliary tract complications after orthotopic liver transplan-tation. *Clin Transplant* 2004; **18:** 647-53.

6. Turrion VS, Alvira LG, Jimenez M, *et al.* Management of the biliary complications associated with liver transplanta-tion: 13 years of experience. *Transplant Proc* 1999; **31:** 2392-3.

7. Guichelaar MM, Benson JT, Malinchoc M, *et al.* Risk factors for and clinical course of non-anastomotic biliary strictures after liver transplantation. *Am J Transplant* 2003; **3:** 885-90.

8. Rizk RS, McVicar JP, Emond MJ, *et al.* Endoscopic management of biliary strictures in liver transplant recipients: effect on patient and graft survival. *Gastrointest Endosc* 1998; **47:** 128-35.

9. Buis CI, Hoekstra H, Verdonk RC, Porte RJ. Causes and consequences of ischemic-type biliary lesions after liver transplantation. *J Hepatobiliary Pancreat Surg* 2006; **13:** 517-24.

10. Sheng R, Zajko AB, Campbell WL, Abu-Elmagd K. Biliary strictures in hepatic transplants: prevalence and types in patients with primary sclerosing cholangitis vs those with other liver diseases. *Am JRoentgenol* 1993; **161:** 297-300.

11. Distante V, Farouk M, Kurzawinski TR, *et al.* Duct-to-duct biliary reconstruction following liver transplantation for primary sclerosing cholangitis. *Transplint* 1996; **9:** 126-30.

12. Feith MP, Klompmaker IJ, Maring JK, *et al.* Biliary recon-struction during liver transplantation in patients with primary sclerosing cholangitis. *Transplant Proc* 1997; **29:** 560-1.

13. Schmitz V, Neumann UP, Puhl G, *et al.* Surgical complica-tions and long-term outcome of different biliary recon-structions in liver transplantation for primary sclerosing cholangitis-choledochoduodenostomy versus choledocho-jejunostomy. *Am JTransplant* 2006; **6:** 379-85.

14. Colonna JO, Shaked A, Gomes AS, *et al.* Biliary strictures complicating liver transplantation. Incidence, pathogen-esis, management, and outcome. *Ann Surg* 1992; **216:** 344-50.

15. Halme L, Hockerstedt K, Lautenschlager I. Cytomegalo-virus infection and development of biliary complications after liver transplantation. *Transplantation* 2003; **75:** 1853-8.

16. Kowdley KV, Fawaz KA, Kaplan MM. Extrahepatic biliary stricture associated with cytomegalovirus in a liver transplant recipient. *Transplint* 1996; **9:** 161-3.

17. Torras J, Llado L, Figueras J, *et al.* Biliary tract complica-tions after liver transplantation: type, management, and outcome. *Transplant Proc* 1999; **31:** 2406.

18. Maheshwari A, Maley W, Li Z, Thuluvath PJ. Biliary complications and outcomes of liver transplantation from donors after cardiac death. *Liver Transpl* 2007; **13:** 1645-53.

19. Dubbeld J, Hoekstra H, Farid W, *et al.* Similar liver transplantation survival with selected cardiac death donors and brain death donors. *Br J Surg* 2010; **97:** 744-53.

20. Campbell WL, Sheng R, Zajko AB, Abu-Elmagd K, Demetris AJ. Intrahepatic biliary strictures after liver transplanta-tion. *Radiology* 1994; **191:** 735-40.

21. Feller RB, Waugh RC, Selby WS, *et al.* Biliary strictures after liver transplantation: clinical picture, correlates and out-comes. *J Gastroenterol Hepatol* 1996; **11:** 21-5.

22. Ward EM, Kiely MJ, Maus TP, Wiesner RH, Krom RA. Hilar biliary strictures after liver transplantation: cholan-giography and percutaneous treatment. *Radiology* 1990; **177:** 259-63.

23. Sanchez-Urdazpal L, Gores GJ, Ward EM, *et al.* Diagnostic features and clinical outcome of ischemic-type biliary complications after liver transplantation. *Hepatology* 1993; **17:** 605-9.

24. Pascher A, Neuhaus P. Bile duct complications after liver transplantation. *Transpl int* 2005; **18:** 627-42.

25. Buis CI, Verdonk RC, van der Jagt EJ, *et al.* Non-anasto-motic biliary strictures after adult liver transplantation part one: radiological features and risk factors for early versus late presentation. *Liver Transpl* 2007; **13:** 708-1.

26. Rull R, Garcia Valdecasas JC, Grande L, *et al.* Intrahepatic biliary lesions after orthotopic liver transplantation. *Transplint* 2001; **14:** 129-34.

27. Shapiro SD. Matrix metalloproteinase degradation of ex-tracellular matrix: biological consequences. *Curr Opin Cell Biol* 1998; **10:** 602-8.

28. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001; **17:** 463-516.

29. Verspaget HW, Kuyvenhoven JP, Sier CF, van Hoek B. Matrix metalloproteinases in chronic liver disease and liver transplantation. In Lendeckel U, Hooper NM, eds *Proteases in Biology and Disease 5: Proteases in Gastrointestinal Tissues.* Dordrecht, the Netherlands: Springer, 2006; 209-34.

30. Harendza S, Lovett DH, Panzer U, *et al.* Linked common polymorphisms in the gelatinase a promoter are associated with diminished transcriptional response to estrogen and genetic fitness. *J Biol Chem* 2003; **278:** 20490-9.

31. 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:** 718-25.

32. Price SJ, Greaves DR, Watkins H. Identification of novel, functional genetic variants in the human matrix metallo-proteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. *J Biol Chem* 2001; **276:** 7549-58.

33. Miao X, Yu C, Tan W, *et al.* A functional polymorphism in the matrix metalloproteinase-2 gene promoter (-1306C/T) is associated with risk ofdevelopment but not metastasis of gastric cardia adenocarcinoma. *Cancer Res* 2003; **63:** 3987-90.

34. Wu J, Zhang L, Luo H, *et al.* Association of matrix metalloproteinases-9 gene polymorphisms with genetic susceptibility to esophageal squamous cell carcinoma. *DNA Cell Biol* 2008; **27:** 553-7.

35. Zhang B, Ye S, Herrmann SM, *et al.* Functional polymorph-ism in the regulatory region of gelatinase B gene in relation to severity ofcoronary atherosclerosis. *Circulation* 1999; **99:** 1788-94.

36. Ten Hove WR, de Rooij BJF, van Hoek B, *et al.* MMP-2 and MMP-9 serum levels change but their gene promotor polymorphisms are not associated with late phase I/R injury or rejection after orthotopic liver transplantation. *Open Transplant Journal* 2008; **2:** 66-72.

37. Zhou LM, Wang L, Palais R, Pryor R, Wittwer CT. High-resolution DNA melting analysis for simultaneous muta-tion scanning and genotyping in solution. *Clin Chem* 2005; **51:** 1770-7.

38. Kubben FJ, Sier CF, Meijer MJ, *et al.* Clinical impact of MMP and TIMP gene polymorphisms in gastric cancer. *Br J Cancer* 2006; **95:** 744-51.

39. Ye S, Dhillon S, Ke X, Collins AR, Day IN. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Res* 2001; **29:** E88-8.

40. Meijer MJ, Mieremet-Ooms MA, van Hogezand RA, *et al.* Role of matrix metalloproteinase, tissue inhibitor of metal-loproteinase and tumor necrosis factor-alpha single nu-cleotide gene polymorphisms in inflammatory bowel disease. *World J Gastroenterol* 2007; **13:** 2960-6.

41. Sanchez-Urdazpal L, Gores GJ, Ward EM, *et al.* Ischemic-type biliary complications after orthotopic liver transplan-tation. *Hepatology* 1992; **16:** 49-53.

42. Buis CI, Geuken E, Visser DS, *et al.* Altered bile composi-tion after liver transplantation is associated with the devel-opment of nonanastomotic biliary strictures. *J Hepatol* 2009; **50:** 69-79.

43. Geuken E, Visser D, Kuipers F, *et al.* Rapid increase of bile salt secretion is associated with bile duct injury after human liver transplantation. *J Hepatol* 2004; **41:** 1017-25.

44. Milani S, Herbst H, Schuppan D, *et al.* Differential expression of matrix-metalloproteinase-1 and -2 genes in normal and fibrotic human liver. *Am J Pathol* 1994; **144:** 528-37.

45. Kuyvenhoven JP, Verspaget HW, Gao Q, *et al.* Assessment of serum matrix metalloproteinases MMP-2 and MMP-9 after human liver transplantation: increased serum MMP-9 level in acute rejection. *Transplantation* 2004; **77:** 1646-52.

46. Benyon RC, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. *Semin Liver Dis* 2001; **21:** 373-84.

47. Sankary HN, McChesney L, Hart M, Foster P, Williams J. Identification of donor and recipient risk factors associated with nonanastomotic biliary strictures in human hepatic allografts. *Transplant Proc* 1993; **25:** 1964-7.

48. Brandsaeter B, Schrumpf E, Bentdal O, *et al.* Recurrent primary sclerosing cholangitis after liver transplantation: a magnetic resonance cholangiography study with analyses of predictive factors. *Liver Transpl* 2005; **11:** 1361-9.