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Genetic polymorphisms of matrix metalloproteinase 3 in primary sclerosing cholangitis

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Abstract

Background—The damaging cholestasis inherent to primary sclerosing cholangitis (PSC) results from bile duct stricturing because of progressive fibrosis. The matrix metalloproteinase 3 (MMP3) degrades a wide range of matrix components and is expressed by activated liver stellate cells, and so is a candidate for involvement with the fibrotic processes underlying PSC. Moreover, the MMP3 gene harbours polymorphisms associated with variation in its activity directly impacting clinical phenotypes.

Aims—We aimed to examine the influence of MMP3 polymorphisms on PSC risk and progression.

Methods—Nine single nucleotide polymorphisms (SNPs) tagging the common genetic variation of MMP3 were genotyped in 266 PSC patients and 407 controls. SNPs and inferred haplotypes were assessed for PSC association by logistic regression and score tests. The effect of SNPs on survival to liver transplant or death was analysed using Cox regression, and Kaplan–Meier curves were constructed.

Results—No association of PSC with individual SNPs or haplotypes of MMP3 was detected. However, progression to death or liver transplant was significantly associated with homozygosity for minor alleles of rs522616, rs650108 and rs683878, particularly among PSC patients with concurrent ulcerative colitis (UC) (strongest in redundant SNPs rs650108/rs683878, hazard ratio = 3.23, 95% confidence interval 1.45–7.25, $P = 0.004$).

Conclusions—Genetic variation in MMP3 influences PSC progression, possibly in the context of coexisting UC. While the functional variants and specific mechanisms remain unknown, this finding implicates the turnover of the extracellular matrix as an important and variable component of PSC pathogenesis. Efforts to understand this process could form the basis for developing effective treatments, which are currently lacking for PSC.

Keywords

cholestasis; genetics; genomics; liver; PSC

Primary sclerosing cholangitis (PSC) is a chronic disease of the liver typified by lymphocytic portal tract inflammation and stricturing of the bile ducts because of concentric obliterative fibrosis. This results in progressive cholestasis leading to cirrhosis and end-stage liver disease (1, 2). The natural history of PSC is variable, although it is generally diagnosed in the fourth decade and the average time from diagnosis to death or liver transplantation, the only effective therapy, is 12–15 years (3). Inflammatory bowel disease (IBD), particularly ulcerative colitis (UC), is frequent in PSC, with some 75–85% of PSC patients having a diagnosis of IBD before or during the course of their disease (4, 5). The epidemiological picture of PSC remains incomplete, but it is certainly a rare disease, with an estimated prevalence in the USA of about one in 7100 individuals (6), consistent with studies from northern Europe (7–9). Despite its rarity, PSC accounts for a substantial percentage of orthotopic liver transplantation (OLT) in the USA (10).

The mechanisms responsible for the development and variable progression of PSC are complex, the net result of the interaction between underlying genetics and environmental exposure (11). While genetic studies of many candidate loci have been performed in PSC, to date, the only convincing genetic associations reported are with extended human leucocyte antigen haplotypes in the major histocompatibility complex region of chromosome 6 [reviewed in (3, 12, 13)]. However, it should be noted that many previous studies were underpowered because of a small sample size, and a number of these loci deserve further study in PSC. Indeed, well-designed candidate gene studies utilizing modestly sized but highly characterized cohorts of PSC patients and controls will continue to be valuable despite the impending prospect of genome-wide studies from international collaborative groups.

The matrix metalloproteinases (MMPs) are prime candidates for harbouring genetic variants potentially contributing to the development or the variable progression of PSC as they actively degrade the extracellular matrix, playing a major role in the modulation of portal-based fibrosis. Among these is MMP3, which is of particular interest to the study of PSC, as it has consistently been shown to be upregulated in inflamed colonic mucosa, contributing to tissue damage in IBD (14–17), is expressed in the liver by activated hepatic stellate cells and has likely antifibrotic activity because of its wide substrate specificity and ability to activate latent proforms of other MMPs (18, 19). Interestingly, MMP3 harbours a well-characterized insertion/deletion variant in its promoter characterized by the presence of either five or six adenine residues, commonly referred to as 5A/6A (rs3025058). The 5A allele has been shown to result in higher MMP3 expression because of disrupted binding of a nuclear factor kappa B dimer, which acts as a transcriptional repressor (20–22), and shows signs of recent positive selection in northern European populations (23). Two previous studies have examined this polymorphism in PSC patients, the first of which reported an association of the MMP3 5A allele and the 5A/5A genotype with PSC and noted increased 5A/5A presence in PSC patients with documented portal hypertension (24). The second study was larger, and detected no such MMP3 association with PSC, but did identify an increased frequency of the 5A/5A genotype in PSC patients with UC compared with those without (25). Both of these studies focused only on the 5A/6A polymorphism of MMP3 and neither provided an analysis of the possible effect on progression to end-stage disease. Thus, the goal of our study was to more fully assess the potential effects of genetic variation in MMP3 on the development and progression of PSC.

Patients and methods

Study participants

The participants in this study, 266 well-documented PSC patients and 407 outpatient-clinic-based controls, were previously recruited into the PSC Resource of Genetic Risk,

Environment and Synergy Studies (PROGRESS), which was created with the aim to uncover the genetic and environmental contributors to PSC pathogenesis. PSC diagnosis was made or confirmed by a qualified physician based on standard PSC criteria, minimally two of the following three criteria being present: (a) cholangiographical evidence of PSC by endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography, percutaneous or intra-operative cholangiogram; (b) evidence of biochemical cholestasis of more than a 6-month duration; and (c) liver biopsy consistent with PSC in the absence of secondary sclerosing cholangitis. Patients fulfilling criteria (b) and (c) but with negative cholangiographical findings (i.e. likely small duct PSC) were excluded. Controls were recruited from the Mayo Clinic Division of General Internal Medicine during annual visits for preventative medical examination. Control exclusion criteria included evidence of prior or current liver disease. The demographical characteristics of the patient and control groups are shown in Table 1. As there is a strong overlap between PSC and IBD, the disease characteristics of PSC patients were also calculated based on the presence and type of IBD, as shown in Table 2.

Informed consent was obtained from all the study participants. PROGRESS and the present study conform to the ethical guidelines of the 1975 Declaration of Helsinki, and have been approved by the Institutional Review Board of Mayo Clinic.

Sample handling and DNA preparation

The collection of blood specimens from the study participants was performed using bar-coded mail-in kits. Isolation of genomic DNA from the blood samples was performed by the Biospecimens Accessioning and Processing laboratory at the Mayo Clinic using the PureGene kit (Gentra Systems, Minneapolis, MN, USA) as specimens were received. Subsequent handling of the DNA, including quality control and dilution to working concentrations, was carried out in our own laboratory.

SNP selection and typing

Haplotype tagging SNPs for MMP3 were identified by means of the linkage disequilibrium (LD)-select method (26) using the CEU population data from HapMap (27). SNPs 5 kb upstream and downstream of MMP3 were included in the selection process, which was based on gene and SNP coordinates from NCBI build 36 and dbSNP build 127, using an r^2 value of 0.9 and a minor allele frequency of 5%. A total of eight bins were identified, of which six were singletons (i.e. contained individual SNPs). A single SNP was chosen to represent each of the two additional bins, with care taken to include the nonsynonymous coding SNP rs679620. Additionally, the 5A/6A insertion/deletion polymorphism located in the promoter (rs3025058) was included in the genotyping panel. A schematic of the MMP3 gene indicating the locations of the tagging SNPs is provided in Figure 1.

All nine polymorphisms of MMP3 were genotyped by TaqMan allelic discrimination assays (Applied Biosciences, Foster City, CA, USA) using the Applied Biosciences 7300 real-time polymerase chain reaction systems.

Statistical analysis

Disease and phenotype association with individual SNPs was assessed using logistic regression to determine odds ratios, 95% confidence intervals (CI) and statistical significance. Haplotypes were inferred using the expectation-maximization algorithm and studied for association with PSC using a score test and simulated P -values using the R package HAPLO.STATS (28). The association of individual SNPs with survival was performed using Cox regression to determine statistical significance along with hazard ratios (HR) and 95% CI; modelling assumptions were checked. Kaplan–Meier curves were also constructed.

Disease-related death and liver transplantation were considered as events in this analysis. In all instances age was included as an adjustment factor, analyses were two-sided, considered multiple inheritance models, and were completed using *SAS* (SAS, Cary, NC, USA) and *R*. To account for multiple testing, we used the procedure proposed by Li and Ji (29), which considers LD between the genotyped SNPs, to calculate an experiment- wise significance level of 0.009 (type I error rate of 5%). Thus, in the results and discussion sections, we will consider *P*-values of 0.05–0.009 to be of ‘borderline significance’ and *P*-values of < 0.009 to be ‘significant’; nominal *P*-values are reported.

Results

Individual SNPs and haplotypes of MMP3 do not contribute to risk of PSC and are not associated with IBD status in the PSC patients

The frequencies of all genotyped MMP3 polymorphisms and the inferred common haplotypes (i.e. combined haplotype frequency greater than 5%) from the 266 PSC patients and 407 controls are shown in Table 3. Statistical analysis identified no association between PSC and MMPs polymorphisms, including the 5A/6A insertion/deletion variant, or the inferred haplotypes. Moreover, analysis considering IBD status, both overall and by IBD subtype, within the PSC patients yielded no significant findings (data not shown). All SNPs conformed to Hardy–Weinberg equilibrium. To further explore the negative findings in the association analysis, we successfully imputed genotypes for seven of the nine studied MMP3 SNPs, including the 5A/6A polymorphism rs3025058, in a German cohort of 1036 UC patients and 1634 controls from existing genome-wide data (30) using a hidden Markov model algorithm implemented in *MACH* V.1.0.16 (31) and the most recent release of 1000 genomes (<http://www.sph.umich.edu/csg/yli/mach/download/1000G-2010-06.html>). No significant associations were identified in the analysis of the UC patients compared with the PSC patients or the controls.

Homozygosity for MMP3 SNPs rs522616, rs650108, and rs683878 alters disease progression to liver transplantation or death in PSC patients, particularly in those with concurrent ulcerative colitis

Cox regression was used to evaluate the potential role of MMP3 variants in disease progression in PSC. The observation period was defined as either time from diagnosis to OLT/death or time from diagnosis to most recent follow-up in the analysis. Homozygosity for the minor alleles of SNPs rs522616, rs650108 and rs683878, but not the 5A/6A polymorphism (rs3025058), was identified as hazards for progression to OLT or death in the Cox regression. These findings were of borderline significance when all patients were considered, but more significant in terms of HR and *P*-values in the subgroup of PSC patients with evidence of concurrent UC (Fig. 2). As a note, the rs650108 and rs683878 genotypes were found to be in strong LD ($r^2 = 0.97$) and completely redundant, except for in one PSC patient who had ‘G/G’ and ‘C/G’ genotypes for the respective SNPs; thus, the results under the recessive model for these two SNPs are presented together. Kaplan–Meier plots illustrating the detrimental effect of recessive carriage of the rs522616 ‘C’ and rs650108/rs683878 ‘A’/‘G’ alleles on event-free survival in all PSC patients and in those with concurrent UC are shown in Figure 2. In addition to looking at the SNPs individually in the Cox regression, we used a stepwise selection process to determine whether multiple SNPs, age at diagnosis or sex made a simultaneous contribution to disease progression. In all cases, after the rs650108 SNP was in the model, no other model was significant. To investigate whether or not the association of MMP3 SNPs with disease progression in PSC was influenced by the development of cholangiocarcinoma (CCA), which was noted in 23 of the 72 progressing patients (all CCA patients were in the progressing group), the Cox analysis was repeated excluding those patients with CCA. No large differences in the *P*-

values were noted when patients with CCA were excluded (e.g. rs650108/rs683878 $P = 0.004$ in PSC with UC analyses both with and without the CCA patients).

Discussion

We have shown the association of genetic variants in the MMP3 gene with an increased rate of progression to end-stage disease in PSC. This phenomenon was particularly notable in PSC patients with concurrent UC, suggesting the possibility that the underlying colitis in these patients may contribute to the influence of MMP3 polymorphisms on disease progression. Perhaps inflammation of the colonic mucosa that occurs in UC creates a milieu in the liver of PSC patients, which promotes liver fibrogenesis and diminished degradation of the extracellular matrix by MMP3, leading to progression of the hepatic disease. Also of note, none of the studied MMP3 variants, including the functional 5A/6A promoter polymorphism, were found to be statistically associated with a risk of PSC or UC in the general sense or with the presence or type of IBD within PSC patients, suggesting that genetic variation in MMP3 alone has little influence on PSC development.

Interestingly, the three alleles found to be associated with progression to end-stage disease are all isolated on haplotypes containing the ancestral 6A allele of the 5A/6A polymorphism rs3025058 (see Table 3 for haplotype structures). This includes three haplotypes containing the associated rs650108 'A' and rs683878 'G' variants, which are nearly redundant, as well as the implicated 'C' allele of rs522616 that is in strong LD with rs650108/rs683878 ($r^2 = 0.70$), and isolated to two of the rs650108/rs683878 'A/G' haplotypes. Considering the configuration of these haplotypes, the finding that the strongest statistical association with the rate of disease progression to end-stage was with the rs650108/rs683878 alleles and the recessive nature of the allelic effect, it seems likely that the functional polymorphism(s) responsible for this increased rate would be in tight linkage with the implicated alleles. Additional mapping in a larger patient population will be required to confirm the observed association and identify the functional allele(s).

The idea that MMP3 could be involved in the pathogenesis of PSC is not novel. Indeed, two studies investigating the 5A/6A polymorphism in PSC patients have been reported (24, 25). The first of these, by Satsangi *et al.* (24), utilized a two-stage design with a total of 111 PSC patients, 84 UC controls and 171 normal subjects. The frequency of the 5A allele was found to be significantly elevated in PSC patients compared with both the UC affected and the normal controls, indicating that MMP3 might influence PSC development. Moreover, this study noted the association of 5A homozygosity with documented portal hypertension in the PSC patients, suggesting a possible influence of MMP3 on disease severity, but no investigation into other PSC severity-related phenotypes was reported (24). The second study of MMP3 in PSC, performed by Wiencke *et al.* (25), was larger, with a total of 165 PSC patients, 118 UC controls and 346 normal subjects. Analysis did not confirm the previously reported MMP3 association with PSC. Although subset analysis did identify an increased frequency of the 5A allele in PSC patients with UC compared with PSC patients without UC, this allele was not associated with CCA, raising doubts about its contribution to disease severity (25). Although we did not note an increased frequency of the 5A allele in PSC patients with UC, our present investigation supports the findings of the latter study in that MMP3 genetic variation does not appear to contribute significantly to PSC development (25). However, our study is not large enough to definitively exclude MMP3 genetic variation as a potential risk for PSC or to examine its effect on the development of CCA. Moreover, our imputation analyses indicate no association of the MMP3 variants (including the 5A/6A allele) with UC in accordance with the previous studies. Finally, as with any investigation into the genetic underpinnings of disease progression, potential bias explicit to disease observation such as variable presentation driving imprecision in the date of PSC

onset and inconsistent listing times and eligibility for transplantation, as well as statistical concerns stemming from testing multiple variants exist and could impact our findings.

The vast majority of previous investigations into the influence of MMP3 genetic variation on a wide array of clinical diseases and phenotypes, including those for PSC (24, 25), have focused on the 5A/6A deletion polymorphism (rs3025058) located in the promoter. Our study is the most comprehensive assessment of the effect of MMP3 variants on disease affecting the liver performed to date. This prior focus on a single polymorphism is the result of strong support for functionality at the 5A/6A site, with convincing evidence for stronger promoter activity with the derived 5A allele (20–22), which appears to have a direct *in vivo* effect illustrated by associations with a number of clinical phenotypes involving imbalanced matrix degradation (32–37). However, considering the large number of features influencing MMP3 gene expression and function (38), other common genetic variants may well demonstrate a clinical effect. This phenomenon was demonstrated recently for the MMP3 neighbour gene MMP1, which also contains a commonly studied functional promoter polymorphism (1G/2G) implicated in various disease processes but was found to have a large percentage of the variation of its serum level in healthy adults accounted for by upstream polymorphisms (39).

In conclusion, while the nature of the association between the identified MMP3 polymorphisms and progression to end-stage PSC cannot currently be explained, our findings suggest that genetic variants in addition to the often-studied 5A/6A promoter polymorphism could have consequences on MMP3 function that impact complex phenotypes. Moreover, the acknowledged role of MMP3 in mediating the degradation of extracellular matrix implies a plausible functionality behind its association with disease progression in PSC and implicates the control of matrix homeostasis as a potentially important player in PSC pathogenesis. Endeavours to better understand the impact of interplay between the numerous genes responsible for these processes could result in improved prediction of PSC progression and possibly lead to the development of effective treatments, which are currently lacking for this disease.

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Fig. 1.

Matrix metalloproteinase 3 (MMP3) gene structure and location of haplotype tagging single nucleotide polymorphisms (SNPs). Haplotype tagging SNPs across the MMP3 gene utilized in this study are as follows: SNP1, rs3025058 (5A/6A); SNP2, rs522616; SNP3, rs3025090; SNP4, rs679620; SNP5, rs476762; SNP6, rs3025066; SNP7, rs650108; SNP8, rs683878; SNP9, rs527832.

Fig. 2.

Kaplan-Meier plots of matrix metalloproteinase 3 (MMP3) rs522616 and rs650108 effects on event-free survival in primary sclerosing cholangitis (PSC) patients. Homozygosity for the minor alleles of rs522616 and rs650108 is significantly associated with progression to orthotopic liver transplantation (OLT) or disease-related death in PSC patients. The strongest association is with the rs650108 'A/A' genotype in PSC patients with coexistent ulcerative colitis (UC) (D). (A) rs522616 in all PSC patients, Cox HR = 2.50, 95% CI 1.08–5.77, $P = 0.032$; (B) rs522616 in PSC patients with UC, Cox HR = 3.23, 95% CI 1.27–8.17, $P = 0.013$; (C) rs650108 in all PSC patients, Cox HR = 2.36, 95% CI 1.08–5.77, $P = 0.022$; (D) rs650108 in PSC patients with UC, Cox HR = 3.26, 95% CI 1.46–7.31, $P = 0.004$.

*rs650108 is redundant with rs683878. CI, confidence interval; HR, hazard ratio.

Table 1

Demographics of primary sclerosing cholangitis patients and controls

	PSC	Control
<i>n</i>	266	407
Sex–Male	59.4%	21.6%
Race–Caucasian	100%	100%
Age at study [*]	52.3 (10.7–85.2)	61.7 (23.5–87.7)
Age at PSC diagnosis [*]	41.4 (8.0–77.0)	–
Duration of PSC [*]	11.0 (0–42.7)	–
IBD	80.8%	Not assessed
Ulcerative colitis [†]	82.3%	
Crohn's disease [†]	10.2%	
Indeterminate colitis [†]	7.4%	
OLT	27.1%	0%
Cholangiocarcinoma	8.6%	0%

^{*} Value expressed as mean (range) in years.

[†] Value as percentage of PSC–IBD patients.

IBD, inflammatory bowel disease; OLT, orthotopic liver transplantation; PSC, primary sclerosing cholangitis.

Table 2

Primary sclerosing cholangitis disease characteristics by inflammatory bowel disease type

	Ulcerative colitis	Crohn's disease	Indeterminate colitis	No IBD
<i>n</i>	177	22	16	51
Sex–Male	61.6%	36.4%	56.3%	62.7%
Age at study*	51.1 (10–85)	50.1 (23–78)	57.3 (19–82)	55.7 (23–81)
Age at PSC diagnosis*	39.5 (8–75)	38.8 (14–58)	46.5 (15–64)	47.4 (15–77)
Duration of PSC*	11.7 (0–43)	11.4 (4–27)	10.8 (2–21)	8.4 (0–28)
Age at IBD diagnosis*	29.7 (3–68)	33.7 (11–58)	40.3 (16–76)	–
Duration IBD before PSC*	9.7 (– 26–51)	5.1 (– 13–29)	6.6 (– 15–28)	–
IBD diagnosis before PSC	69.0%	50.0%	57.1%	–
IBD diagnosis concurrent PSC	17.8%	31.8%	7.1%	–
IBD diagnosis after PSC	13.2%	18.2%	35.7%	–
OLT	27.1%	27.3%	25.0%	27.5%
Cholangiocarcinoma	10.2%	9.1%	0.0%	5.9%

* Value expressed as Mean (range) in years.

IBD, inflammatory bowel disease; OLT, orthotopic liver transplantation; PSC, primary sclerosing cholangitis.

Matrix metalloproteinase 3 single nucleotide polymorphisms and haplotypes in primary sclerosing cholangitis patients and controls

Table 3

		Frequency										
		SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	PSC	CON
Hap 1	5A	T	T	G	T	T	T	G	C	G	0.466	0.430
Hap 2	6A	T	T	G	C	A	T	G	C	G	0.111	0.124
Hap 3	6A	C	C	G	C	T	T	A	G	A	0.087	0.110
Hap 4	6A	C	C	G	C	T	T	A	G	G	0.105	0.090
Hap 5	6A	T	T	A	C	T	T	G	C	G	0.105	0.085
Hap 6	5A	T	T	G	T	T	C	G	C	G	0.079	0.085
Hap 7	6A	T	T	G	C	T	T	A	G	G	0.045	0.068
PSC*	0.45	0.19	0.11	0.45	0.11	0.11	0.08	0.24	0.24	0.09		
CON*	0.48	0.20	0.09	0.48	0.12	0.12	0.09	0.27	0.27	0.11		

* Minor allele frequency; SNP1, rs3025058; SNP2, rs522616; SNP3, rs3025090; SNP4, rs679620; SNP5, rs476762; SNP6, rs3025066; SNP7, rs650108; SNP8, rs683878; SNP9, rs527832. CON, control; MMP3, matrix metalloproteinase 3; PSC, primary sclerosing cholangitis; SNP, single nucleotide polymorphism.