

BRIEF ARTICLE

## Analysis of *monocyte chemotactic protein-1* gene polymorphism in patients with spontaneous bacterial peritonitis

Erwin Gäbele, Marcus Mühlbauer, Hartwig Paulo, Monika Johann, Christin Meltzer, Franz Leidl, Norbert Wodarz, Reiner Wiest, Jürgen Schölmerich, Claus Hellerbrand

Erwin Gäbele, Marcus Mühlbauer, Hartwig Paulo, Christin Meltzer, Franz Leidl, Reiner Wiest, Jürgen Schölmerich, Claus Hellerbrand, Department of Internal Medicine I, University Medical Center Regensburg, D-93042 Regensburg, Germany  
Monika Johann, Norbert Wodarz, Department of Psychiatry, University Medical Center Regensburg, D-93042 Regensburg, Germany

**Author contributions:** Gäbele E and Mühlbauer M contributed equally to this study; Hellerbrand C designed the study; Gäbele E collected the data; Mühlbauer M, Paulo H, Johann M, Meltzer C, Leidl F, Wodarz N, Gäbele E, Hellerbrand C, Wiest R and Schölmerich J analyzed and interpreted the data; Gäbele E and Hellerbrand C drafted the article and all other authors revised it critically for important intellectual content; all authors approved the final version.

**Correspondence to:** Claus Hellerbrand, MD, Department of Internal Medicine I, University Medical Center Regensburg, D-93042 Regensburg,

Germany. [claus.hellerbrand@klinik.uni-regensburg.de](mailto:claus.hellerbrand@klinik.uni-regensburg.de)

Telephone: +49-941-9447155 Fax: +49-941-9447154

Received: April 27, 2009 Revised: August 18, 2009

Accepted: August 25, 2009

Published online: November 28, 2009

### Abstract

**AIM:** To investigate a genetic polymorphism of the *monocyte chemotactic protein-1* (MCP-1) gene in patients with spontaneous bacterial peritonitis (SBP).

**METHODS:** MCP-1 genotyping was performed in 23 patients with SBP and 83 cirrhotic control patients with non-infected ascites.

**RESULTS:** The frequency of carriers of the G-allele was lower in SBP patients but this difference did not reach statistical significance. However, in the subgroup of patients with alcoholic cirrhosis ( $n = 80$ ), carriers of the G-allele were significantly less frequent in SBP-patients (38.1%) than in cirrhotic controls (67.8%,  $P = 0.021$ ).

**CONCLUSION:** In patients with alcoholic liver cirrhosis, the -2518 MCP-1 genotype AA is a risk factor for the development of SBP.

**Key words:** Monocyte chemotactic protein-1; Chemokines; Spontaneous bacterial peritonitis; Polymorphism; Liver cirrhosis

**Peer reviewer:** Dr. Yogesh K Chawla, Professor, Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Gäbele E, Mühlbauer M, Paulo H, Johann M, Meltzer C, Leidl F, Wodarz N, Wiest R, Schölmerich J, Hellerbrand C. Analysis of *monocyte chemotactic protein-1* gene polymorphism in patients with spontaneous bacterial peritonitis. *World J Gastroenterol* 2009; 15(44): 5558-5562 Available from: URL: <http://www.wjgnet.com/1007-9327/15/5558.asp> DOI: <http://dx.doi.org/10.3748/wjg.15.5558>

### INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a common and potentially life-threatening complication in patients with cirrhosis. It is a prototypical infective disease in cirrhotic patients characterized by peritoneal neutrophil infiltration, which also serves as a diagnostic criterion for SBP (e.g. an ascites neutrophil count greater than  $250/\text{mm}^3$ )<sup>[1]</sup>.

Factors influencing the development of SBP in patients with liver cirrhosis are poorly understood. Previous studies have indicated that peritoneal macrophages of cirrhotic patients might contribute to the control of SBP or influence its associated pathology in human cirrhosis by producing high quantities of angiogenic peptides and nitric oxide<sup>[2-5]</sup>. Accordingly, elevated concentrations of proinflammatory cytokines are found in ascites of patients<sup>[6,7]</sup>.

Therefore, it is of particular interest to identify mechanisms underlying the recruitment and activation of macrophages/monocytes that infiltrate the ascites of cirrhotic patients. Chemotactic cytokines are known to be critical mediators of inflammatory cell trafficking into sites of injury and are crucial for the modulation of tissue injury, inflammation, and repair.

Monocyte chemotactic protein-1 (MCP-1) is one of the most potent chemokines for monocytes/macrophages and activated lymphocytes during infections<sup>[8]</sup>. In addition, several studies have shown that neutrophil infiltration is affected either directly or indirectly *via* MCP-1<sup>[9,10]</sup>.

Several lines of evidence indicate that MCP-1 might play a role in the recruitment and maintenance of the inflammatory infiltrate during liver injury. MCP-1 secretion is up-regulated during chronic hepatitis and correlates with the severity of hepatic inflammation<sup>[11-13]</sup>.

Furthermore, a previous study showed elevated MCP-1 levels in ascites of cirrhotic patients as compared to controls<sup>[14]</sup>. Moreover, during SBP, even higher MCP-1 levels were measured in ascites suggesting that this potent chemokine plays a pathophysiological role during the development and course of SBP.

Recently, a mutation in the distal regulatory region of the MCP-1 gene at position -2518 relatively to the transcription start site (based on the published sequence, Gene Bank accession number D26087) was identified (A-2518G)<sup>[15]</sup>. *In vitro* stimulated monocytes from individuals carrying a G-allele at -2518 produced more MCP-1 than cells from A/A homozygous subjects<sup>[15]</sup>. Several further studies have confirmed the functional effect of this single nucleotide polymorphism (SNP)<sup>[13,16-18]</sup>.

The prevalence of high MCP-1 producing genotypes has been shown to be associated with increased susceptibility and severity of several diseases, including bronchial asthma<sup>[19]</sup>, premature kidney graft failure<sup>[20]</sup>, Crohn's disease<sup>[21]</sup>, cutaneous vasculitis<sup>[22]</sup>, and tuberculosis<sup>[16]</sup>. Furthermore, this genetic polymorphism has been shown to be a risk factor for the progression of chronic viral hepatitis C infection<sup>[13]</sup>.

The aim of this study was to analyze the association of this functional promoter SNP of the MCP-1 gene with SBP.

## MATERIALS AND METHODS

### Patients and controls

We studied 106 patients with liver cirrhosis and ascites. Clinical charts of patients diagnosed with chronic liver disease at the medical department of the University of Regensburg during the period 2001-2007 were studied retrospectively. Based on the availability of DNA samples in an established tissue bank, patients were included in the study. Liver carcinoma, other neoplasms or HIV-infection were exclusion criteria. Clinical charts revealed diagnosis of SBP in 23 cases, while no SBP had ever occurred in 83 patients. In the majority of patients, chronic alcohol abuse was the cause of the underlying liver disease (Table 1). In the other patients, chronic hepatitis B or C infection and hemochromatosis were the cause of cirrhosis. Patients were considered to have alcohol related cirrhosis if alcohol intake had been in excess of 100 g/d for more than 10 years and if tests for viral, metabolic, and immune etiologies were negative. Patient characteristics are summarized in Table 1.

As an additional control group, 118 heavy drinkers without evidence of liver damage (90 male, 28 female; mean age: 42.5 ± 9.1 years) were analyzed. These unrelated individuals of German descent were recruited from an in-patient abstention program at the Department of Psychiatry of the University of Regensburg. Each

**Table 1** Characteristics of cirrhotic patients with SBP and cirrhotic control patients with ascites (mean ± SD)

	Control cirrhotic patients (n = 83)	Cirrhotic patients with SBP (n = 23)
Age (yr)	55.8 ± 10.5	58.7 ± 8.6
Male gender (%)	75.9	78.3
Albumin (g/dL)	3.0 ± 0.6	3.0 ± 0.6
Prothrombin activity (%)	63.8 ± 22.1	54.9 ± 20.8
Bilirubin (mg/dL)	4.9 ± 6.3	4.9 ± 5.9
Chronic alcohol abuse as etiology of cirrhosis (%)	71.1	91.3

SBP: Spontaneous bacterial peritonitis.

subject met the criteria of alcohol dependence according to ICD-10 alcohol dependence according to DSM-IV (American Psychiatric Association, 1994) and ICD-10 (World Health Organization, 1992).

At the day of admission, several serum parameters were analyzed, including transaminases (alanine aminotransferase, aspartate aminotransferase,  $\gamma$  glutamyltransferase), and anti hepatitis B virus (HCV)- and hepatitis B virus (HBV)-antibodies. Previous and current drinking habits, family history of non-alcoholic liver diseases, and potential previous complications of advanced liver damages, such as ascites or esophageal varices, were examined. Furthermore, within 14 d after admission a clinical and ultrasound examination of the abdomen was performed. Only alcohol-dependent subjects with an alcohol consumption of at least 100 g/d for more than 5 years were included. Furthermore, clinical or sonomorphological indications for advanced liver damage and serological evidence of HBV or HCV infection were exclusion criteria.

Patients and controls were Caucasians and their geographical origin was Southern Germany. Informed consent was obtained from all patients and the study was approved by the local ethics committee.

### DNA isolation and MCP-1 genotyping

DNA isolation and MCP-1 genotyping were performed as described previously<sup>[13,23]</sup>. Briefly, genomic DNA specimens were prepared from 200  $\mu$ L blood using the QIAamp blood kit, following the manufacturer's instructions (Qiagen, Hilden, Germany). The G to A polymorphisms at position -2518 of the MCP-1 gene was analyzed by performing PCR and restriction fragment length polymorphism analysis. PCR was performed under standard conditions (35 cycles, annealing temperature: 55°C) in a total reaction volume of 50  $\mu$ L containing 2  $\mu$ L of diluted genomic DNA, using the following pair of primers: forward: 5'-CCGAGATGTTCCCAGCACAG-3' and reverse: 5'-CTGCTTTGCTTGTGCCTCTT-3'. PCR products were digested by *Pvu*II, and the resulting fragments were separated by electrophoresis in a 2% agarose gel and visualized by ethidium bromide staining. With the -2518 A polymorphic base, the recognition sequence 5'-CAG/CTG-3' is modified to 5'-CAG/CTA-3', which is not cut by *Pvu*II.

Table 2 Frequencies of -2518 MCP-1 genotypes *n* (%)

-2518 MCP-1 genotype	Cirrhotic patients with ascites ( <i>n</i> = 106)	Patients with alcoholic cirrhosis and ascites ( <i>n</i> = 80)	Heavy drinkers w/o cirrhosis ( <i>n</i> = 118)
A/A	45 (42.5)	32 (40.0)	64 (54.2)
A/G	51 (48.1)	39 (48.8)	47 (39.8)
G/G	10 (9.4)	9 (11.3)	7 (5.9)

### Statistical analysis

Results are expressed as mean  $\pm$  SD (range) or, when indicated, as absolute number and percent. Genotype frequencies are reported with their group percentages. Contingency table analysis and two-sided Fisher's exact tests were used for comparison of qualitative variables.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Characteristics of the study population

The clinical and epidemiological characteristics of SBP patients and cirrhotic control patients are summarized in Table 1. There was no statistical difference between patients with SBP and non-infected cirrhotic patients with ascites with respect to age, distribution of sexes, and serum laboratory findings, such as albumin, bilirubin, or prothrombin activity. However, the percentage of patients with chronic excessive alcohol abuse as the cause of liver cirrhosis was significantly higher in the SBP group (91.3% *vs* 71.1% in the cirrhosis group,  $P = 0.046$ ), which agreed with previous studies that showed greater susceptibility to infections, especially SBP, in alcohol-induced liver cirrhosis<sup>[24]</sup>.

### Frequency of the -2518 MCP-1 polymorphism in patients and controls

The frequencies of the different MCP-1 genotypes in our cohort of patients with liver cirrhosis and ascites are summarized in Table 2. In addition, genotype distribution in the subgroup of patients with chronic excessive alcohol consumption as an underlying cause of liver disease and cirrhosis is depicted. Furthermore, MCP-1 genotypes were analyzed in heavy drinkers without evidence of liver damage.

The frequency of individual genotypes in the group of heavy drinkers without cirrhosis was similar to those previously reported in other Caucasian control populations<sup>[19,20,22,25]</sup>. Genotype distribution did not differ significantly between the whole group of cirrhotic patients with ascites and the subgroup of patients with alcohol related cirrhosis. However, irrespective of the underlying cause of liver disease, the genotype AA was less frequent in cirrhotic patients with and without ascites (40.0% and 42.5%, respectively) as compared to heavy drinkers without evidence of liver damage (54.2%) although these differences did not reach statistical significance ( $P = 0.084$  and  $P = 0.060$ , respectively).

Most previous *in vitro* and epidemiological studies reported functional and disease-related differences between

Table 3 Frequencies of carriers of the G-allele of the -2518 MCP-1 promoter polymorphism in patients with SBP and cirrhotic control patients with ascites *n* (%)

-2518 MCP-1 genotype	Whole cohort ( <i>n</i> = 106)		Alcoholic cirrhosis ( <i>n</i> = 80)	
	No SBP ( <i>n</i> = 83)	SBP ( <i>n</i> = 23)	No SBP ( <i>n</i> = 59)	SBP ( <i>n</i> = 21)
A/G or G/G	52 (62.7)	9 (39.1)	40 (67.8)	8 (38.1)
A/A	31 (37.3)	14 (60.9)	19 (32.2)	13 (61.9)

-2518 MCP-1 genotypes AA and non-AA (i.e. carriers of the G-allele: genotypes AG and GG); therefore, we continued this differentiation throughout the following analysis and focused on the comparison of patients with no G-allele (AA-homozygotes) and carriers of the G-allele (genotypes AG and GG).

### Frequency of the -2518 MCP-1 polymorphism in patients with and without SBP

In our cohort of 106 patients with ascites, SBP has been diagnosed in 23 individuals (21.7%), while in 83 cirrhotic patients (78.3%), despite mostly long lasting episodes of ascites, no SBP had occurred. The frequency of carriers of the G-allele was lower in the SBP group but this difference did not reach statistical significance [9/23 (39.1%) *vs* 52/83 (62.7%),  $P = 0.057$ , Table 3]. Also, in the subgroup of patients with alcoholic cirrhosis (*n* = 80), carriers of the G-allele were less frequent in SBP-patients compared to patients without SBP, and in this case, the difference reached statistical significance [8/21 (38.1%) *vs* 40/59 (67.8%),  $P = 0.021$ , Table 3].

## DISCUSSION

The aim of this study was to analyze the association of the functional MCP-1 promoter polymorphism (A-2518G) with SBP. Interestingly, in patients with alcoholic liver cirrhosis, the genotype AA was significantly more frequent in SBP-patients than in cirrhotic control patients with ascites only. Previously, this MCP-1 genotype has been shown to be associated with reduced MCP-1 release of monocytes *in vitro* and MCP-1 tissue levels *in vivo*, respectively<sup>[13,16]</sup>.

Furthermore, in a previous study<sup>[14]</sup>, MCP-1 levels in ascites were significantly higher when compared with their levels in serum, suggesting a chemotactic gradient towards the peritoneal cavity, even in the absence of infection. This chemotactic gradient could be operative in the chemotaxis of monocytes/macrophages. Antigenic stimuli drive these cells to synthesize further proinflammatory cytokines and chemokines, and thus might also modify the systemic response to the infection. Furthermore, the chemotactic MCP-1 gradient might directly or indirectly favor leukocyte migration towards the peritoneal cavity, even in the absence of infection. We could speculate that in patients with SBP, higher MCP-1 ascites fluid levels are implicated in the infiltration of the peritoneal cavity. Furthermore, in several experimental models of acute peritonitis, high concentrations of MCP-1 have

been shown to have beneficial effects by recruiting macrophages and neutrophils to the site of infection<sup>[26-28]</sup>. Taken together these data indicate that, in patients with alcoholic liver cirrhosis, the -2518 MCP-1 genotype AA is a risk factor for the development of SBP, possibly *via* reduced MCP-1 ascites levels as compared to patients carrying the G-allele.

Interestingly, only in the subgroup of patients with chronic excessive alcohol abuse as the underlying cause of liver cirrhosis was the association between MCP-1 genotype and SBP found. In a previous study, patients with alcohol induced cirrhosis and patients with other hepatopathies showed similar MCP-1 serum and ascitic fluid levels<sup>[14]</sup>.

Notably, carriers of the G-allele of the MCP-1 polymorphism were more frequent in patients with alcohol induced cirrhosis than in heavy drinkers without evidence of liver damage. This difference did not reach statistical significance, but taken together with our previous finding that carrying the -2518 MCP-1 G-allele is a risk factor for disease progression in patients with chronic hepatitis C infection<sup>[13]</sup>, these data indicate that this functional SNP also affects fibrosis progression in patients with alcoholic liver disease. Thus, the same SNP appears to affect two related pathophysiological mechanisms (namely development of cirrhosis and SBP) but to different extents, depending on the underlying liver disease. Inflammatory and immune responses are altered in cirrhotic patients in general<sup>[29,30]</sup>; however, it has to be considered that there are differences between the underlying liver diseases leading to liver cirrhosis. This might be one explanation why it is more difficult to identify an association with the functional MCP-1 polymorphism and SBP in patients with liver disease other than alcoholic liver disease. Furthermore, in patients with alcoholic cirrhosis, the mean endotoxin concentration was significantly higher than in patients with non-alcoholic cirrhosis<sup>[31]</sup>, and bacterial translocation and endotoxin concentration have been shown to play an important role in the pathophysiology of SBP development<sup>[32]</sup>. In addition, it has been shown that LPS downregulates the specific MCP-1 receptor, CCR2, and abolishes macrophage infiltration in an animal model of acute infection<sup>[33]</sup>. Therefore, we speculate that higher LPS levels in patients with alcoholic liver disease are the reason why an association between the -2518 MCP-1 polymorphism and SBP was found only in the subgroup of patients with this underlying liver disease.

Finally, as in most genetic association studies, it has to be considered that the SNP investigated is in linkage disequilibrium to a different disease-associated genetic variation. Theoretically, this potential genetic variation might particularly affect the pathophysiology of alcoholic but not viral or other liver diseases. However, the high levels of MCP-1 in ascites in patients suffering from SBP and the functional relevance of the -2518 MCP-1 polymorphism demonstrated in several studies, argue against this hypothesis.

In summary, this is one of the first genetic association studies in patients with SBP. Our data indicate that the -2518 MCP-1 genotype AA is a risk factor for the

development of SBP in patients with alcoholic cirrhosis. Of course, our results have to be confirmed in a larger cohort of patients with independent and prospective studies. However, it is intriguing to speculate that genotyping might be helpful to identify cirrhotic patients with a higher risk of developing this life threatening complication and to apply prophylactic treatment specifically to this subset of patients.

## COMMENTS

### Background

Spontaneous bacterial peritonitis (SBP) is a potentially life threatening complication in patients with liver cirrhosis and ascites, caused by peritoneal neutrophil infiltration. However, factors leading to enhanced neutrophil infiltration of the peritoneal cavity with consecutive SBP are poorly characterized.

### Research frontiers

Monocyte-chemotactic protein-1 (MCP-1) is an important chemokine for several proinflammatory cells, and there exists a functional single nucleotide polymorphism (SNP) of the MCP-1 promoter (A-2518G) leading to higher MCP-1 expression in carriers of the G-allele. In the present study, the frequency of this MCP-1 SNP was analyzed in patients with SBP and liver cirrhosis.

### Innovations and breakthroughs

Accumulating evidence suggests that genotypes leading to increased MCP-1 production contribute to susceptibility and severity of diseases such as asthma, kidney graft failure, Crohn's disease, cutaneous vasculitis, tuberculosis, and chronic hepatitis C infection. This is the first genetic association study in patients with SBP to show that the -2518 MCP-1 genotype AA is a risk factor to development of SBP in patients with alcoholic cirrhosis.

### Applications

Genotyping of the -2518 MCP-1 SNP in cirrhotic patients might be beneficial to identify patients with increased risk for the development of SBP. Thus, early antibiotic treatment of such patients could help to decrease the mortality of this severe complication in liver cirrhosis.

### Terminology

SBP is an infection of the ascites in patients with liver cirrhosis, diagnosed by a neutrophil count greater than 250/mm<sup>3</sup> in the ascitic fluid. MCP-1 is one of the most potent chemokines for the recruitment of inflammatory cell-types during infection. For example, MCP-1 is upregulated in chronic hepatitis.

### Peer review

This retrospective study identified an association of a genetic polymorphism of the chemokine MCP-1 with increased risk to develop SBP in patients with alcohol-related liver cirrhosis. This is one of the first genetic association studies in patients with SBP and the results might be of high clinical relevance.

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S- Editor Li LF L- Editor Stewart GJ E- Editor Zheng XM