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A variant in the *nuclear dot protein 52 kDa* gene increases the risk for spontaneous bacterial peritonitis in patients with alcoholic liver cirrhosis

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ABSTRACT

Background: Spontaneous bacterial peritonitis is frequently a fatal infection in patients with liver cirrhosis. We investigated if *nuclear dot protein 52 kDa* (*NDP52*), a negative regulator of toll-like receptor (TLR) signalling and autophagy adaptor protein, might be involved.
Methods: Two cohorts comprising 152 (derivation cohort) and 198 patients (validation cohort) with decompensated liver cirrhosis and 168 healthy controls were genotyped for the rs2303015 polymorphism in the *NDP52* gene and prospectively followed-up for spontaneous bacterial peritonitis.
Results: Overall, 57 (38%) patients in the derivation cohort and 77 (39%) in the validation cohort had spontaneous bacterial peritonitis. Cirrhosis was due to alcohol abuse in 57% of the derivation and 66% of the validation cohort. In patients with alcoholic cirrhosis, patients with spontaneous bacterial peritonitis had an increased frequency of the *NDP52* rs2303015 minor variant in the derivation ($p = 0.04$) and in the validation cohort ($p = 0.01$). Multivariate analysis confirmed this minor variant (odds ratio 4.7, $p = 0.002$) and the *TLR2* −16934 TT variant (odds ratio 2.5, $p = 0.008$) as risk factors for spontaneous bacterial peritonitis. In addition, presence of the *NDP52* minor variant affected survival negatively.
Conclusion: Presence of the *NDP52* rs2303015 minor variant increases the risk for spontaneous bacterial peritonitis in patients with alcoholic cirrhosis.

1. Introduction

Bacterial infections are a major cause of morbidity and mortality in patients with liver cirrhosis [1]. Next to urinary tract infections spontaneous bacterial peritonitis (SBP) is the most frequent infection in these patients [2]. SBP is a primary form of peritonitis and occurs almost exclusively in patients with liver cirrhosis. It is defined as bacterial peritonitis occurring in the absence of a contiguous source of infection (e.g. bowel perforation) [3]. According to international guidelines, diagnosis is made if the polymorphonuclear (PMN) cell count in ascites exceeds 250 cells/μl [4,5]. Causative bacteria can only be identified in around 40% of cases by conventional culture of ascites fluid [6]. Despite treatment with antibiotics and albumin, mortality has remained high, ranging from 16 to 67% [1,6,7]. Since bacterial antibiotic resistance has risen for the last years [8,9], empirical treatment is difficult. Recurrence of SBP is common [10–12].

Primary antibiotic prophylaxis can prevent SBP reliably in selected patients [4]. Therefore, better understanding of the mechanisms leading to SBP and recognizing patients at particularly high risk might be an effective approach to decrease mortality. In general, SBP is thought to occur via bacterial translocation from the intestine [13]. However, bacterial translocation is common in patients with liver cirrhosis [14] but results in SBP only in a

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Table 1
Clinical characteristics of patients and controls.

	Derivation cohort		Validation cohort		Healthy controls
	All patients	Patients with alcoholic cirrhosis	All patients	Patients with alcoholic cirrhosis	
Total number	152	87	198	131	168
Median age (years)	56 (23–86)	56 (29–81)	61 (28–94)	62 (36–94)	36 (20–67)
Male gender	110 (72)	62 (71)	135 (68)	89 (68)	101 (60)
Aetiology of cirrhosis					
Alcohol	87 (57)	87 (100)	131 (66)	131 (100)	–
Chronic hepatitis B/C	17 (11)		12 (6)		–
Alcohol and chronic hepatitis B/C	7 (5)		9 (5)		–
Cryptogenic liver cirrhosis	15 (10)		10 (5)		–
Primary sclerosing cholangitis	4 (3)		5 (3)		–
Others	20 (13)		31 (16)		–
INR	1.3 (0.9–4.7)	1.3 (0.9–4.7)	1.3 (0.9–3.8)	1.3 (0.9–3.8)	–
Total serum bilirubin (mg/dL)	2.1 (0.24–38.79)	2.0 (0.24–38.79)	2.0 (0.1–36.0)	2.0 (0.3–36.0)	–
Serum creatinine (mg/dL)	1.3 (0.38–14.99)	1.3 (0.38–14.93)	1.4 (0.29–8.0)	1.4 (0.29–6.7)	–
Serum albumin (g/L)	26.4 (10.4–45.9)	26.3 (13.7–40.3)	27.9 (6.9–47.8)	27.7 (6.9–47.8)	–
Ascites albumin (g/L)	5.7 (0.7–29.7)	5.9 (1.1–23.7)	5.3 (0.1–28.0)	5.4 (0.1–28.0)	–
MELD score	16 (6–43)	15 (6–40)	17 (6–43)	16 (6–43)	–
Child–Pugh score A/B/C (%)	6/44/50	6/44/50	4/49/47	2/51/47	–
Proton pump inhibitors	115 (76)	67 (77)	168 (85)	118 (90)	–
SBP (%)	57 (38)	28 (32)	77 (39)	50 (38)	–
Previous/during follow-up	18 (32)/39 (68)	9 (32)/19 (68)	33 (43)/44 (57)	22 (44)/28 (56)	–
Rifaximin (%)	0 (0)	0 (0)	11 (5.6)	9 (6.9)	–

Continuous variable expressed as median (range).
INR, international normalized ratio; MELD, Model of End-Stage Liver Disease; SBP, spontaneous bacterial peritonitis.

subgroup of patients. Gastrointestinal bleeding, use of proton pump inhibitors, low ascites protein content, genetic polymorphisms and prior SBP have been reported as risk factors for SBP [15].

Recently, a missense mutation in the *nuclear dot protein 52 kDa* (*NDP52*) gene, which leads to the replacement of valine by alanine at position 248, has been associated with Crohn's disease [16]. *NDP52* is an adaptor protein for selective autophagy expressed in many cell types. It binds to bacteria coated with ubiquitin or to galectin 8, which is exposed if vacuoles contain bacteria, and induces autophagy [17,18]. In addition, *NDP52* has been reported to regulate TLR-signalling negatively [19]. When HeLa cells were transfected with the minor variant of the *NDP52* rs2303015 polymorphism, this negative feedback was lost [16].

Here, we studied if the *NDP52* rs2303015 polymorphism might be a genetic risk factor for SBP. We genotyped two large cohorts of patients at risk for SBP for this polymorphism and the other reported genetic risk factors for SBP located in the *nucleotide-binding oligomerization domain-containing 2* (*NOD2*) [20,21], the *toll-like receptor 2* (*TLR2*), the *farnesoid X receptor* (*FXR*, *NR1H4*) [22] and the *monocyte chemotactic protein-1* (*MCP1*) [23,24] genes.

2. Patients and methods

2.1. Patients

Blood samples and clinical data from patients with liver cirrhosis who received a paracentesis were collected prospectively at the Department of Internal Medicine I from 2006 to 2007 (derivation cohort, results of this cohort have been published previously [20,25]) and from 2012 to 2013 (validation cohort). Diagnosis of liver cirrhosis was based on histology, or on specific clinical, laboratory and radiological evidence of portal hypertension in patients with chronic liver disease. Diagnostic paracentesis was performed when indicated by international guidelines [4]. SBP was diagnosed according to these guidelines when PMN counts exceeded 250 cells/μl [4]. All patients were followed up as long as they were seen in our department. End of observation was March 2014, six months after the last patient had been included. To limit false classification of patients into the SBP group, we performed an analysis based on combined retrospective and prospective occurrence of SBP (cumulative occurrence) and on prospective occurrence of SBP alone, excluding patients with reported prior SBP.

Table 2
Distribution of genotype frequencies of the *nuclear dot protein 52 kDa* rs2303015 polymorphism in patients and controls.

	GG	GA	AA
Derivation cohort	132	20	0
Validation cohort	181	16	1
Healthy controls	150	18	0

Blood samples collected from 168 healthy blood donors were used to analyse the normal distribution of the genotypes. The study protocol adhered to the ethical guidelines of the Helsinki Declaration and was approved by the local ethics committee. Written informed consent was obtained prior to inclusion in this study.

2.2. Determination of the FXR, TLR2, MCP-1, NOD2 and NDP52 genotypes

Genomic DNA was extracted from 200 μl EDTA-blood using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany).

Determination of the *NDP52* rs2303015 and the *MCP-1* rs1024611 single nucleotide polymorphism was performed by LightCycler real time PCR using LightSNiP (SimpleProbe) assays from TIB-MolBiol (Berlin, Germany). Samples were set up in a final volume of 10 μl, containing 1 μl of DNA solution, 5 μl of Fermentas Maxima Probe qPCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA), and 0.5 μl of LightSNiP reagent mix (Tib MolBiol, Berlin, Germany). The cycling conditions were chosen according to the manufacturer's protocol. The other polymorphisms were determined as described previously [22].

2.3. Statistical analysis

Wilcoxon–Mann–Whitney-*U* test was used for analysis of quantitative data. Fisher's exact test was applied to analyse qualitative