Common *NOD2* Risk Variants as Major Susceptibility Factors for Bacterial Infections in Compensated Cirrhosis

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OBJECTIVES: **Common nucleotide-binding oligomerization domain containing 2 (*NOD2*) gene variants have been associated with bacterial infections (BIs) in cirrhosis, in particular, spontaneous bacterial peritonitis, and mortality. Our aim was to evaluate the independent association of *NOD2* variants with BI according to the decompensation stage.**

METHODS: **Consecutive patients with cirrhosis in 2 academic medical centers were included and genotyped for the *NOD2* variants p.R702W, p.G908R, and c.3020insC. Electronic medical records were screened for BI (requiring antibiotic therapy) and past and present decompensation (as defined by variceal bleeding, encephalopathy, ascites, and/or jaundice). Clinically significant portal hypertension (CSPH) was assessed with liver stiffness and/or hepatic venous pressure gradient measurements.**

RESULTS: **Overall, 735 patients were recruited (men 65%; interquartile age range 53–68 years). Alcoholic cirrhosis was the predominant etiology (n = 406, 55%), and most patients were in the decompensated stage (n = 531, 72%). In total, 158 patients (21%) carried at least one *NOD2* variant. BIs were detected in 263 patients (36%), and *NOD2* variants were associated with BI (odds ratio = 1.58; 95% confidence interval 1.11–2.27; $P = 0.02$). In compensated patients, the combination of *NOD2* variants and presence of CSPH was the best independent predictors of BI, whereas other factors, such as spleen size and hemoglobin, and decompensations including hepatic encephalopathy or jaundice, gained relevance in decompensated patients.**

CONCLUSIONS: ***NOD2* risk variants are associated with BI in cirrhosis. The genetic effect on BI is strongest in compensated patients, whereas in decompensated patients their presence is less relevant. In this situation, CSPH becomes an independent factor associated with BI.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A2

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INTRODUCTION

Cirrhosis, the common end stage of many chronic liver diseases, is the eighth cause of years of life lost because of premature mortality in the United States (1). In the past decade, further insight into the natural history of cirrhosis was achieved, and 2 distinct stages of the disease with prognostic relevance were identified, namely, compensated and decompensated cirrhosis (2–7). The median survival of compensated patients, those who have never had clinically evident complications of cirrhosis, is greater than a decade (2), whereas patients in the decompensated stage, as defined by the presence of actual or previous variceal bleeding (VB), ascites, hepatic encephalopathy (HE), and/or jaundice, are the ones at highest risk of dying from their liver disease. Bacterial infections (BIs) play a significant role in the natural history of cirrhosis, leading to a dramatic increase in mortality (8–10). BI and decompensation are closely intertwined, in the sense that BI precipitate decompensation, or *vice versa*, i.e., that decompensation (e.g., VB) favors the development of BI (11–14).

Variants of the *NOD2* (nucleotide-binding oligomerization domain containing 2) gene were initially associated with impaired mucosal barrier function in Crohn's disease (15,16). NOD2 is an intracellular pattern recognition receptor expressed in macrophages and is involved in the intestinal recognition of bacteria and bacterial products, shaping bacterial colonization (16). Insufficient activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in carriers of *NOD2* risk variants may result in deficient antimicrobial activity, altered microbiome, and enhanced bacterial translocation (BT) from the intestine (17).

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Previous studies have associated *NOD2* gene variants with spontaneous bacterial peritonitis (SBP) and mortality in decompensated cirrhosis (18–20). The association between *NOD2* variants and non-SBP BI according to decompensation stage was evaluated in one study (18), in which no significance ($P = 0.107$) was observed, but the analysis was limited by the small study size (122 compensated and 121 decompensated patients). Furthermore, the definition of decompensation stage used in this study (18) was not the standard one (2–7); instead acute decompensation was defined by the acute development of large ascites, acute HE, VB, and/or the presence of BI at the time of enrollment.

The effects of *NOD2* variants on BI according to the decompensation stage in patients with cirrhosis and their interaction with other risk factors are therefore unknown. Therefore, the aim of the present study was to assess the association between common *NOD2* risk variants (p.R702W, pG908R, and/or c.3020insC) and BI according to decompensation stage in a large cohort of patients with cirrhosis.

METHODS

Study population

Seven hundred and thirty-five patients with cirrhosis from 2 academic medical centers in Homburg and Halle, Germany, were prospectively included between February 2014 and February 2017. All consecutive Caucasian patients with cirrhosis, hospitalized on the wards or attending liver outpatient clinics, were considered for inclusion. Patients with severe comorbidities, such as end-stage heart failure, HIV infection, and nonresectable cancer except hepatocellular carcinoma (Barcelona Clinic Liver Cancer stages A–C), and patients in whom a BI could not be confirmed were excluded (Figure 1). Cirrhosis was defined by (i) biopsy, (ii) a combination of clinical, laboratory, ultrasound, and endoscopy findings, or (iii) transient elastography > 13.0 kPa. Median elastography (n = 422) was 35.3 kPa (interquartile range [IQR] 20.2–55.2 kPa). In patients with transient elastography <19.7 kPa (21), diagnosis of cirrhosis was additionally confirmed by (i) or (ii). The study was conducted according to the Declaration of Helsinki and Good Clinical Practice (European guidelines). Institutional review board approval was obtained (Homburg: 271/11, Halle 2017-85). All participants provided written informed consent. Electronic medical records were reviewed for clinical data, including past and present decompensation and BI. Further information regarding medication use (such as β-blockers, long-term antibiotic therapy, lactulose, and statins) and laboratory parameters at the time of inclusion were recorded.

NOD2 genotyping

After isolation of genomic DNA from EDTA-anticoagulated blood using a membrane-based extraction kit (Qiagen, Hilden, Germany), the *NOD2* variants rs2066844 (p.R702W), rs2066845 (p.G908R), and rs2066847 (c.3020insC) were genotyped using TaqMan polymerase chain reaction (PCR)-based allelic discrimination assays (Life Technologies, Carlsbad, CA). The assays utilized were p.R702W, C__11717468_20; p.G908R, C__11717466_20;

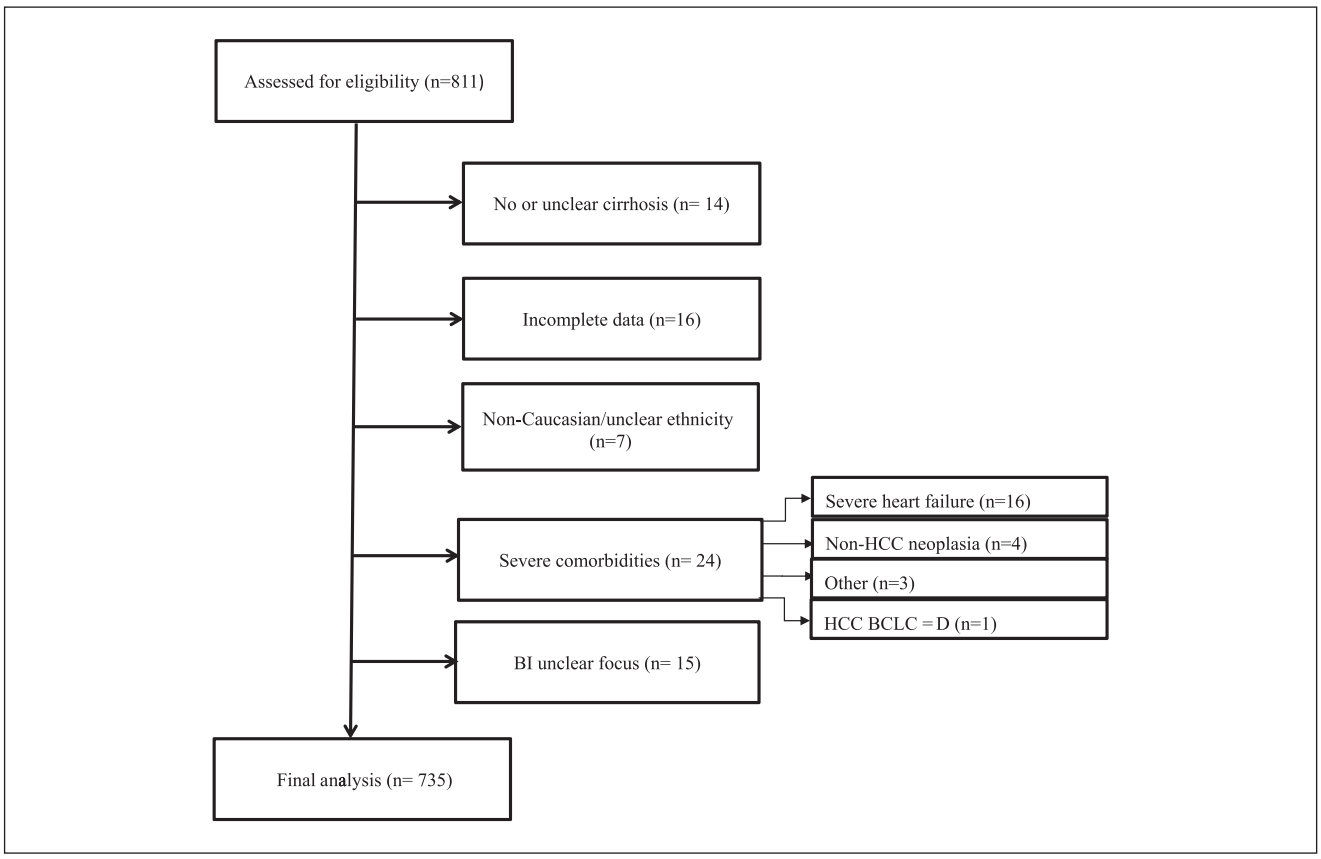

Figure 1. Flow chart outlining inclusion and exclusion of patients in the study

c.3020insC, specifically designed primer, and probe sequences were MGB_F CCAGGTTGTCCAATAACTGCATC; MGB_R CCTTACCAGA-CTTCCAGGATGGT; VIC TGCAGGCCCC TTG; and FAM CTGCAGGCCCTTG. This determination was offered as part of the routine workup after informed consent and was used in the context of prescreening for the randomized controlled Impact of NOD2 genotype-guided antibiotic prevention on survival in patients with liver Cirrhosis and Ascites (INCA) trial (22). All technicians performing the genotyping were blinded to clinical data.

Bacterial infections

All episodes of previous or current BI, which took place after the diagnosis of cirrhosis, were recorded. BI was defined according to the criteria outlined by Bajaj et al. (23). Only infections that were treated with antibiotics were considered as such. Specifically, criteria were as follows: (i) SBP: ascitic fluid was defined as polymorphonuclear cell count >250/μL. (ii) Pulmonary BIs were defined as the presence of an infiltrate/consolidation/cavity plus at least 2 of the following criteria: fever ≥ 38 °C or hypothermia < 35 °C, dyspnoea, or clinical signs of pulmonary BI (cough and purulent sputum, pleuritic chest pain) on physical examination. (iii) Urinary tract infection was defined as > 10 white blood cell (WBC) per high-power field in urine microscopy and positive urine cultures, or significant WBC count in urine (>500/μL) with typical complaints (fever/pain/dysuria/pollakisuria). (iv) Spontaneous bacteremia was defined as growth of a noncommon skin contaminant in blood cultures, without evidence of infection located at another body site (24). When bacteremia was detected in a patient with SBP, pulmonary BI, urinary tract infection, sepsis, or other BI, it was interpreted as secondary to these infections and defined by the primary infection. (e) *Clostridium difficile* colitis was defined as diarrhea with a positive *C. difficile* toxin assay. Sepsis was diagnosed and evaluated separately by the presence of a BI, together with impaired host response and organ dysfunction as described in international consensus criteria (25).

Portal hypertension

In subgroups of patients, hepatic venous pressure gradient (HVPG) (n = 139, 19%), transient elastography (n = 413, 56%), and spleen diameter (n = 655, 89%) measurements were performed. In those patients who had available data, the liver stiffness to spleen/platelet (LSPS) score (n = 379) was calculated. LSPS score >1.72 has a high specificity and sensitivity for the presence of clinically significant portal hypertension (CSPH), as defined by HVPG ≥ 10 mm Hg (3,26). A composite parameter was imputed for the estimation of CSPH using HVPG or LSPS score according to their respective cut-offs, and this estimation was available in 481 (65.4%) patients. In the case that the patients had both measurements with inconsistent results, HVPG was used as the reference standard.

Clinical decompensation

Decompensation was defined by present or past VB, HE, ascites, and/or jaundice, as endorsed by both the American Association for the Study of the Liver Diseases (6) and the European Association for the Study of the Liver (7). This definition has previously been shown to have prognostic relevance (2,4,5). Specifically, VB was considered according to the Baveno definition (3). HE was assessed following the West Haven criteria (27). Ascites was defined by the presence of signs of ascites on physical examination and/or confirmed by abdominal ultrasound examination. Patients without clinical ascites but who were dependent on diuretics to treat ascites were considered as decompensated because of the presence of ascites. Jaundice was defined arbitrarily by a total bilirubin ≥3 mg/dL. According to this definition, patients who had shown past decompensation (e.g., VB) were considered to have reached the decompensated stage of the disease even though at the time of inclusion they had no clinically evident decompensation. Therefore, most patients who were compensated were Child–Pugh A and most patients who were decompensated were Child–Pugh B or C. Nevertheless, it is mathematically possible that Child–Pugh A patients are decompensated (e.g., a patient with a good liver function with only ascites or previous VB) and Child–Pugh B patients are compensated (e.g., patients who have had no decompensation despite an altered liver function with low albumin and high international normalized ratio).

Statistical analyses

All variables are described as proportions, means with s.d., or medians with IQR. Univariate analysis was performed with chi-squared test, *t* test or Mann-Whitney *U* test, according to the distribution of the test variable. The analysis was stratified for the presence of decompensation and CSPH to evaluate interaction and confounding. A second analysis with stratification according to Child–Pugh A vs B and C was also performed. Variables with *P* values <0.1 in the univariate analysis were included in stepwise backward logistic regression multivariate models. Multiplicative interaction was tested in the models by introducing the product of the dependent variables. Different models were constructed to avoid collinearity and overfitting. Collinearity was tested using the variance inflation factor. Even if collinearity was not detected, models were constructed to eliminate redundant information (e.g., model of end-stage liver disease [MELD] and creatinine). Overfitting was avoided by including a maximum of 5 - 10 variables per event. Akaike information criterion (AIC) and Bayesian information criterion were used to evaluate the performance of the models. Given the presence of missing values specifically for CSPH, the corrected AIC was used, in which a correction factor is applied according to the numbers of observations and variables. These criteria take into account not only the fit of the model but also the number of included variables; hence, the most parsimonious model is preferred. The lower the AIC, corrected AIC, or Bayesian information criterion, the better the fit of the model, because it explains more variability of the dependent variable (in our case BI). The statistical analyses were performed with SPSS 22.0 (SPSS, Munich, Germany). Two-sided *P* values <0.05 were regarded as significant. German Clinical Trials Register DRKS00005616 (registered January 22, 2014).

RESULTS

Table 1 summarizes the baseline patient characteristics. The predominant etiology of cirrhosis was alcoholic liver disease (n = 406, 55.2%). Most patients presented with Child–Pugh class A (A: 55.1%, B: 39.6%, and C: 5.3%) at the time of inclusion, and almost 3 quarters of our patient population were in the decompensated stage of the disease (n = 531, 72.2%). The median MELD score was 10.8 (IQR 8.3–14.8). Approximately one fifth of the population (n = 158, 21.5%) were carriers of at least one *NOD2* risk variant. Only 2 patients were homozygous carriers, and 5 patients carried 2 heterozygous variants. Because of the low frequency of these patients, they were combined with the patients who were

Table 1. Baseline characteristics

Parameter	Total (N = 735)
Age (yr)	61 (53–68)
Gender (male)	476 (64.8)
NOD2 risk allele (pos)	158 (21.5)
p.R702W (neg/het/hom)	638 (86.8)/96 (13.1)/1 (0.1)
p.G908R (neg/het/hom)	713 (97.0)/22 (3.0)/0 (0)
c.3020insc (neg/het/hom)	693 (94.3)/41 (5.6)/1 (0.1)
MELD (points)	10.8 (8.3–14.8)
CPS (points)	6 (5–7)
HCC (yes)	89 (12.1)
Varices (yes)	372 (50.6)
Etiology of cirrhosis	
Alcoholic	406 (55.2)
NASH	52 (7.1)
Hepatitis C	106 (14.4)
Hepatitis B	22 (3.0)
Others	76 (10.3)
Cryptogenic	73 (9.9)
Decompensation	531 (72.2)
Ascites (yes)	456 (62.0)
HE (yes)	140 (19.0)
VB (yes)	1,076 (14.4)
Jaundice (yes)	221 (30.1)
Medication	
Betablocker (yes)	365 (49.7)
Long-term antibiotic therapy (yes)	157 (21.4)
Lactulose (yes)	243 (33.1)
Statin (yes)	110 (15.0)
PPI (yes)	498 (67.8)
Laboratory values	
Serum sodium (mmol/L)	138 (135–141)
Creatinine (mg/dL)	0.94 (0.76–1.22)
Total bilirubin (mg/dL)	1.2 (0.7–2.2)
ASAT (U/L)	46 (32.4–72.0)
ALAT (U/L)	31.2 (21.0–50.0)
CRP (mg/dL)	6.8 (2.1–19.3)
Albumin (g/dL)	36.0 (30.1–41.0)
Hemoglobin (g/dL)	12.1 (10.1–14.0)
WBC ($\times 10^9$)	6.2 (4.5–8.3)
Platelets ($\times 10^9$)	135 (89–197)
INR	1.2 (1.1–1.4)
PTT (s)	31.0 (27–36)

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; Ascites, patients treated with diuretics and refractory ascites vs no ascites; BI, bacterial infection; CPS, Child–Pugh Score; CRP, C-reactive protein; HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; het, heterozygous; hom, homozygous; IQR, interquartile range; MELD Score, Model of End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis; PPI, proton pump inhibitor; PTT, partial thromboplastin time; VB, variceal bleeding; WBC, white blood cells. Values are given as median and IQR, or frequencies and percentages.

heterozygous carriers, and further analysis is performed only according to the presence or absence of risk *NOD2* variants. The prevalence of *NOD2* variants was similar in patients with compensated (n = 42, 20.6%) and decompensated cirrhosis (n = 116, 21.8%).

Overall, 263 (35.8%) patients had a previous history of or current BI. BI occurred relatively shortly before inclusion (median 3 [IQR 25/75 0.0–37.0] months). Among those, 85 patients (36.2%) with BI were present at inclusion.

Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/CTG/A2) summarizes the different BIs. Expectedly, patients with decompensated cirrhosis had a higher prevalence of BI (n = 230, 43.3%) than patients with compensated cirrhosis (n = 33, 16.2%). The presence of a *NOD2* gene variant was associated with BI (unadjusted odds ratio [OR] = 1.58; 95% confidence interval [CI] 1.10–2.27). The magnitude of the effect was higher in compensated (unadjusted OR = 2.69; 95% CI 1.19–6.05) than in decompensated patients (unadjusted OR = 1.41; 95% CI 0.94–2.14), indicating the presence of interaction between the stage of the disease and the presence of *NOD2* variants. Similar results, albeit less marked, were observed when the patients were stratified according to Child–Pugh class (A vs B/C) (OR = 1.97; 95% CI 1.16–3.32 vs OR = 1.20; 95% CI 0.72–2.02, respectively). Of our patients, 55 (7.5%) had a previous transjugular intrahepatic portosystemic shunt. Among these patients, no association of *NOD2* variants with BIs could be detected (OR = 0.87; 95% CI 0.24–3.23; $P = 1.0$).

Predictors of BI in compensated patients

Table 2 presents the results of the univariate analysis of BI predictors among compensated patients. Among the different multivariate models (Table 3), including the variables associated with BI in the univariate analysis, the combination of the presence of a *NOD2* risk variant (OR = 4.79; 95% CI 1.51–15.18) and the presence of CSPH (OR = 4.35; 95% CI 1.29–14.66) were the best independent predictors of BI in compensated patients. Among the patients in whom CSPH was estimated, the effect of *NOD2* on BI was smaller among patients with CSPH (n = 69: unadjusted OR = 4.54; 95% CI 1.14–18.1) than in patients without CSPH (n = 64: unadjusted OR = 5.67; 95% CI 0.71–45.5).

Given the fact that the liver disease was slightly more severe in the compensated patients with an estimation of CSPH (as defined by HVPG or LSPS score) as compared to compensated patients without an estimation of CSPH (see Table 2, Supplemental Digital Content 1, http://links.lww.com/CTG/A2), the analysis was repeated in the subgroup of patients who had an estimation of CSPH. Consistently, in this subgroup (n = 133), the models that combined *NOD2* and CSPH were among the best for identifying the patients with BI in our population of compensated cirrhosis, as indicated by the lowest (corrected) AIC (see Table 3, Supplemental Digital Content 1, http://links.lww.com/CTG/A2).

Predictors of BI in decompensated patients

Table 4 summarizes the univariate analysis of predictors of BI in decompensated patients. The presence of *NOD2* variants was not associated to BI in the univariate analysis of this subgroup of patients. Other risk indicators were identified, including the variables that are used to calculate the MELD score (bilirubin, creatinine, and international normalized ratio), markers of inflammation (C-reactive protein and WBC),

Table 2. Univariate analysis of the predictors of BI among compensated patients

Parameter	BI (N = 33)	No BI (N = 171)	*P* value
Age (yr)	65 (58–75)	60 (52–68)	0.06
MELD	8.0 (6.6–9.9)	8.3 (7.2–9.6)	0.44
NOD2 risk allele (yes)	12 (36.4)	30 (17.5)	**0.02**
Gender (male)	19 (57.6)	115 (67.3)	0.32
Diabetes (yes)	15 (45.5)	56 (32.7)	0.17
Alcoholic cirrhosis	13 (39.4)	47 (27.5)	0.21
FibroScan (kPa)	36.6 (17.6–48.2)	30.5 (18.8–54.2)	**0.03**
Spleen size (cm)	13.6 (12.2–15.2)	12.0 (10.6–13.7)	**0.05**
LSPS	2.46 (1.64–4.31)	1.61 (1.07–3.53)	0.33
HPVG (mm Hg)	18.0 (5.5–21.5)	13.0 (7.0–20.5)	0.77
CSPH (yes)	18 (81.8)	53 (43.4)	0.06
PPI (yes)	22 (66.7)	77 (45.0)	**0.04**
Betablocker (yes)	11 (33.3)	64 (37.4)	0.70
Long-term antibiotic therapy (yes)	1 (3.0)	3 (1.7)	0.51
Lactulose (yes)	1 (3.0)	7 (4.1)	1.0
Statin (yes)	11 (33.3)	33 (19.3)	0.10
Serum sodium (mmol/L)	140 (139–142)	140 (138–142)	0.81
Creatinine (mg/L)	0.83 (0.71–1.00)	0.84 (0.72–0.96)	0.97
Bilirubin (mg/dL)	0.8 (0.4–1.2)	0.7 (0.5–1.1)	0.75
ASAT (U/L)	42 (28–60)	46 (31–78)	0.24
ALAT (U/L)	38 (25–46)	39 (28–82)	0.2
CRP (mg/dL)	5.0 (2.0–14.6)	2.2 (0.8–5.3)	0.74
Albumin (g/L)	37 (33–42)	42 (38–45)	**0.001**
Hemoglobin (g/dL)	12.9 (11.6–13.9)	14.2 (12.6–15.3)	**0.001**
Platelets ($\times 10^9$)	175 (128–244)	149 (99–198)	**0.03**
WBC ($\times 10^9$)	5.6 (4.8–7.3)	6.3 (4.8–8.2)	0.25
INR	1.08 (1.01–1.18)	1.10 (1.04–1.21)	0.40
PTT (s)	30 (26–34)	28 (26–31)	**0.003**

BI, bacterial infection; CPS, Child–Pugh Score; CRP, C-reactive protein; CSPH, estimation of clinically significant portal hypertension; MELD Score, Model of End-Stage Liver Disease; OR, odds ratio; PPI, proton pump inhibitor; WBC, white blood cells; CI, confidence interval.
Values are given as median (IQR) or frequencies and percentages.
Significant *P* values are highlighted in bold.

specific decompensations of cirrhosis, such as HE and jaundice, and administration of long-term antibiotic therapy. Given the lack of association on univariate analysis with the variable of interest (i.e., presence of *NOD2* variants) in decompensated patients, no further models were calculated.

DISCUSSION

Bacterial infections cause high morbidity and mortality in patients with cirrhosis (28,29). In this large multicenter study, we demonstrate an association between carriage of a *NOD2* risk allele and BI in patients with cirrhosis. Of note, in patients with compensated disease, the presence of a *NOD2* risk allele has a greater impact on the occurrence of BI than in patients with decompensated cirrhosis. In compensated cirrhosis, the presence of CSPH as assessed by HPVG and LSPS score was another independent variable associated to BI. In contrast, in decompensated cirrhosis, the presence of a *NOD2* variant loses its relevance and other risk indicators gained a more prominent role.

Compensated and decompensated cirrhosis are 2 distinct clinical situations, which are characterized by markedly different prognosis (2). Furthermore, the pathophysiological mechanisms that affect the natural history of the disease also differ between these two disease stages (30–32). As genetic factors only represent one of many drivers in patients with decompensated cirrhosis, genetic risk factors are accordingly more likely to be relevant in compensated cirrhosis. Correspondingly, we confirmed in our cohort the presence of a *NOD2* risk variant as major risk factors for BI in compensated cirrhosis, in addition to proton pump inhibitor use, partial thromboplastin time, hemoglobin levels, platelet count, and the presence of CSPH. In models incorporating multiple significant contributors, the

Table 3. Models to identify the variables independently associated with BI in compensated cirrhosis

Variables introduced	N =	Final model	OR	95% CI	*P* value	AIC	AICc	BIC
NOD2, alb, Hb	195	*NOD2*, Hb	2.51, 0.76	1.07–5.89, 0.63–0.91	0.03, 0.003	145.6	145.7	155.4
NOD2, alb, PTT	192	*NOD2*, alb, PTT	2.50, 0.94, 1.09	1.03–6.10, 0.88–1.00, 1.01–1.18	0.04, 0.05, 0.02	155.2	155.3	168.2
NOD2, alb, platelets	195	*NOD2*, alb, platelets	2.17, 0.91, 1.01	0.88–5.32, 0.85–0.97, 1.00–1.01	0.09, 0.004, 0.003	162.6	162.7	175.7
NOD2, alb, PPI	195	*NOD2*, alb, PPI	2.42, 0.92, 2.19	1.01–5.78, 0.87–0.99, 0.96–5.00	0.05, 0.02, 0.06	138.4	138.5	151.5
NOD2, alb, spleen size	173	Alb	0.91	0.85–0.98	0.01	109.9	109.9	113.2
NOD2, alb, CSPH	132	*NOD2*, CSPH	4.79, 4.35	1.51–15.18, 1.29–14.66	0.008, 0.02	17.8	17.9	26.5
NOD2, Hb, PTT	196	*NOD2*, Hb, PTT	2.53, 0.80, 1.08	1.06–6.08, 0.67–0.97, 1.01–1.17	0.04, 0.02, 0.03	165	165.1	178.1
NOD2, Hb, platelets	200	*NOD2*, Hb, platelets	2.61, 0.75, 1.01	1.09–6.26, 0.62–0.91, 1.00–1.01	0.03, 0.003, 0.005	163.7	163.8	176.9
NOD2, Hb, PPI	201	*NOD2*, Hb	2.51, 0.76	1.08–5.85, 0.64–0.91	0.03, 0.003	146.0	146.1	155.9
NOD2, Hb, spleen size	177	Hb	0.73	0.60–0.88	0.001	138.9	138.9	142.2
NOD2, Hb, CSPH	133	*NOD2*, Hb	4.43, 0.63	1.37–14.36, 0.47–0.84	0.01, 0.002	—	—	—
NOD2, PTT, platelets	195	*NOD2*, PTT, platelets	3.11, 1.12, 1.01	1.27–7.58, 1.04–1.21, 1.00–1.01	0.01, 0.003, 0.002	161.5	161.6	174.6
NOD2, PTT, PPI	196	*NOD2*, PTT, PPI	3.03, 1.11, 2.65	1.27–7.20, 1.03–1.19, 1.16–6.06	0.01, 0.005, 0.02	124.3	124.4	137.4
NOD2, PTT, spleen size	173	*NOD2*, PTT	2.72, 1.13	1.04–7.14, 1.04–1.22	0.04, 0.002	93.1	93.2	102.5
NOD2, PTT, CSPH	132	*NOD2*, PTT, CSPH	5.05, 1.08, 3.43	1.54–16.48, 0.99–1.19, 0.99-11.88	0.007, 0.09, 0.05	72.6	72.8	84.2
NOD2, platelets, PPI	200	*NOD2*, platelets, PPI	3.06, 1.01, 2.21	1.31–7.18, 1.00–1.01, 0.98–5.01	0.01, 0.01, 0.06	165.7	165.8	178.9
NOD2, platelets, spleen size	176	*NOD2*, platelets, spleen size	2.90, 1.01, 1.25	1.13–7.49, 1.00–1.01, 1.05-1.50	0.03, 0.02, 0.02	141.4	141.5	154.1
NOD2, platelets, CSPH	133	*NOD2*, platelets, CSPH	4.82, 1.01, 7.32	1.48–15.73, 1.00–1.02, 1.81-29.61	0.009, 0.06, 0.005	93.9	94.1	105.4
NOD2, PPI, spleen size	180	*NOD2*, spleen size	2.57, 1.18	1.02–6.46, 1.00–1.39	0.05, 0.05	125.4	125.5	134.9
NOD2, PPI, CSPH	133	*NOD2*, CSPH	4.86, 4.30	1.53–15.39, 1.27–14.48	0.007, 0.02	—	—	—
NOD2, spleen size, CSPH	133	*NOD2*, CSPH	4.83, 4.24	1.52–15.29, 1.26–14.32	0.007,0.02	—	—	—

AIC, Akaike information criterion; alb, albumin; BIC, Bayesian information criterion; AICc, corrected Akaike information criterion; CSPH, clinically significant portal hypertension variable; Hb, hemoglobin; MELD Score, Model of End-Stage Liver Disease; NOD2, carriage of a NOD2 risk allele (p.R702W, p.G908R, and c.3020insC); OR, odds ratio; PTT, partial thromboplastin time; CI, confidence interval.
When the same model was repeatedly identified, the calculation of AIC, AICc, and BIC was done with the subset which included the greatest number of patients.

model consisting of the presence of at least one *NOD2* risk variant and CSPH was most accurate.

In decompensated cirrhosis, as previously demonstrated (10,29) and also confirmed in our cohort, additional factors reflecting the degree of liver injury (MELD score, liver stiffness, albumin, partial thromboplastin time, and platelet count), the presence of complications (including ascites, HE, and jaundice) and markers of portal hypertension (i.e., spleen size) are additionally relevant (8).

The functional mechanisms that link the presence of a *NOD2* risk variant to BI susceptibility in cirrhosis have yet to be fully defined. One might hypothesize that the development of a leaky gut in patients with cirrhosis (33) is favored by *NOD2* risk variants, as the presence of a *NOD2* risk allele has been associated to BT (33). In decompensated cirrhosis, other factors additionally impair the intestinal mucosal barrier, and these could override in magnitude the predisposition caused by the presence of *NOD2* risk variants.

Table 4. Univariate analysis of the predictors of BI among decompensated patients

Parameter	BI (N = 230)	No BI (N = 301)	*P* value
Age (yr)	61 (52–68)	60 (54–69)	0.88
MELD	14 (10–19)	12 (9–15)	**<0.001**
NOD2 risk allele (yes)	58 (25.2)	58 (19.3)	0.11
Gender (male)	154 (67.0)	188 (62.5)	0.31
Diabetes (yes)	70 (30.4)	81 (26.9)	0.38
Alcoholic cirrhosis	157 (68.3)	189 (62.8)	0.20
HE (yes)	79 (34.3)	61 (20.3)	**<0.001**
Ascites (yes)	204 (88.9)	252 (83.7)	0.13
Jaundice (yes)	119 (51.7)	100 (33.2)	**<0.001**
FibroScan (kPa)	43.5 (26.5–69.1)	46.0 (26.9–67.8)	0.77
Spleen size (cm)	14.0 (12.0–15.4)	13.0 (11.4–14.7)	**0.004**
PPI (yes)	184 (0.80)	215 (71.4)	**0.03**
Betablocker (yes)	120 (52.2)	170 (56.5)	0.33
Long-term antibiotic therapy (yes)	91 (39.6)	62 (20.6)	**<0.001**
Lactulose (yes)	111 (48.3)	124 (41.2)	0.11
Statin (yes)	27 (11.7)	39 (13.0)	0.69
Serum sodium (mmol/L)	137 (133–140)	138 (135–140)	0.11
Creatinine (mg/L)	1.11 (0.83–1.46)	0.96 (0.77–1.24)	**<0.001**
Bilirubin (mg/dL)	1.6 (0.9–3.2)	1.3 (0.8–2.5)	**0.03**
ASAT (U/L)	52 (35.4–75)	45 (32–67)	0.34
ALAT (U/L)	26 (19–40)	31 (22–45)	0.52
CRP (mg/dL)	14.9 (5.5–41.5)	6.8 (2.4–18.4)	**<0.001**
Albumin (g/L)	32 (27–37)	35 (30–39)	**<0.001**
Hemoglobin (g/dL)	10.6 (8.9–12.4)	11.8 (10.1–13.6)	**<0.001**
Platelets ($\times 10^9$)	120 (78–188)	134 (88–194)	0.92
WBC ($\times 10^9$)	6.5 (4.4–9.6)	6.2 (4.4–7.7)	**0.008**
INR	1.28 (1.15–1.52)	1.20 (1.10–1.36)	**<0.001**
PTT (s)	33 (30–39)	31 (27–35)	**<0.001**

CPS, Child–Pugh Score; CRP, C-reactive protein; CSPH, clinically significant portal hypertension variable; HE, hepatic encephalopathy; LSPS, liver stiffness to spleen/platelet score; MELD Score, Model of End-Stage Liver Disease; OR, odds ratio; PPI, proton pump inhibitor; WBC, white blood cells; CI, confidence interval.
Values are given as median or frequencies and percentages.
Significant *P* values are highlighted in bold.

Incorporating the recent systemic inflammation hypothesis (34), increased inflammation due to an increase of pathogen-associated molecular patterns and damage-associated molecular patterns might be paramount for patients with compensated cirrhosis, with presence of *NOD2* risk variants and few other risk factors. In this sense, a recently published longitudinal study identified C-reactive protein as an independent predictor of decompensation in patients with compensated cirrhosis (35). Notwithstanding the limitations of the small subgroup size, the impact of *NOD2* risk variants on BI was even more pronounced among those patients with compensated disease who did *not* present with CSPH, providing further support for this hypothesis.

This is the first study that evaluates the association between the carriage of a *NOD2* risk variant and the spectrum of BI in cirrhosis according to decompensation stage. A previous smaller study, including 243 cases with cirrhosis, failed to confirm an association of non-SBP BI with *NOD2* risk variants (18), although a trend was observed in Kaplan–Meier analysis. Even though in this study both compensated and decompensated patients were included, the definition of decompensation used in this study, and the other currently available studies, is not the one that has been shown to have prognostic relevance (2). Previous studies have focused on the role of *NOD2* in decompensated stage of cirrhosis and development of SBP with controversial results. On one hand, an association between *NOD2* variants and all cases of SBP (19,20) or culture-positive SBP (20) was found; on the other hand, another study failed to replicate this association (33), albeit increased BT was found in carriers of the p.G908R variant in this cohort. In our cohort, although we could not confirm the association between carriage of a *NOD2* risk allele and SBP, we did observe an increased frequency of

spontaneous bacteremia (see Table 1, Supplemental Digital Content 1, http://links.lww.com/CTG/A2), which shares a common pathophysiology with SBP (24).

Our study has limitations mainly inherent to its cross-sectional nature: First, the BIs were identified retrospectively. To minimize bias, we applied the strict definitions of BI that have been previously described and included only patients with verified BI, which took place after the diagnosis of cirrhosis. Furthermore, clinical records were systematically screened, including all microbiology reports and corresponding medical records. Second, not all patients had an estimation of CSPH, which was based either on HVPG measurements in a large number of patients, the gold standard for determining the presence of CSPH, or the LSPS score developed by Berzigotti et al. (26). The latter approach has shown to have high accuracy for the detection of CSPH. Therefore, we combined both parameters to estimate the presence of CSPH. Of note, within the group of compensated patients with an available estimation of CSPH, liver disease was slightly more advanced than in patients without these measurements. To address this issue, a sensitivity analysis was performed, and the results could be replicated in the subgroup of patients with an available estimation of CSPH, confirming the same predictors for BI as in the whole group. Finally, due to the design of the study, cause-effect associations cannot be strictly assumed. Indeed, the required temporal association between the independent variables and the effect (BI) can only be definitely assured for the presence of the inherited *NOD2* variants.

In conclusion, our results highlight that the common *NOD2* risk variants are a major susceptibility factor for BI in the compensated stage of cirrhosis. The combination of *NOD2* variants and CSPH is the best model indicating risk of BI in this stage of the disease, whereas in decompensated cirrhosis traditional markers of liver failure are stronger risk indicators. Further studies are warranted to evaluate how early intervention strategies could improve the outcome of patients at highest risk.

CONFLICTS OF INTEREST

Guarantor of the article: Frank Lammert, MD, PhD.

Specific author contributions: M.C.R. and C.R. are co-first authorship. A.Z. and F.L. have co-senior authorship. M.C.R., C.R., and F.L. designed the study; M.C., R.G., E.V., F.G., and B.A. participated in the acquisition of clinical data, drafted the manuscript, and together with M.C.R., C.R., A.Z., and F.L. analyzed the data and finalized the manuscript, which was then revised by all authors. The final draft of the manuscript has been approved by all authors. The contents of this manuscript are our original work and have not been published, in whole or in part, prior to or simultaneous with our submission of the manuscript.

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Study Highlights

WHAT IS KNOWN

- ✓ The common *NOD2* gene variants p.R702W, p.G908R, and c.3020insC are susceptibility factors for SBP in decompensated cirrhosis.
- ✓ Assessment of the impact of *NOD2* variants according to the decompensation stage of cirrhosis and the influence of markers of portal hypertension is lacking.

WHAT IS NEW HERE

- ✓ The presence of common *NOD2* gene variants is a risk factor for BIs in cirrhosis in general.
- ✓ The genetic effect is strongest in compensated cirrhosis and also dependent on the presence of CSPH, whereas in decompensated cirrhosis, traditional factors associated with liver failure dominate.

TRANSLATIONAL IMPACT

- ✓ The analysis of increased bacterial translocation and/or altered gut microbiota are of great interest for further studies and might reveal further causal pathways.
- ✓ Further studies are warranted to evaluate early (molecular) intervention strategies to improve the outcome of patients at highest risk.

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