

RESEARCH ARTICLE

Open Access



Genotype–phenotype associations of polymorphisms within the gene locus of NOD-like receptor pyrin domain containing 3 in Swiss inflammatory bowel disease patients

Priyatharsan Yoganathan¹, Jean-Benoit Rossel², Sebastian Bruno Ulrich Jordi^{1,3}, Yannick Franc², Luc Biedermann¹, Benjamin Misselwitz^{1,3}, Martin Hausmann¹, Gerhard Rogler^{1,4}, Michael Scharl^{1,4} and Isabelle Frey-Wagner^{1,5,6*}  Swiss IBD cohort study group

Abstract

Background: Genetic variations within the regulatory region of the gene encoding NOD-like receptor pyrin domain containing 3 (*NLRP3*) have been associated with Crohn's Disease (CD). *NLRP3* is part of the *NLRP3*-inflammasome that mediates the maturation of IL-1 β and IL-18. Carrying the major allele of the single nucleotide polymorphisms (SNPs) rs10733113, rs4353135 and rs55646866 is associated with an increased risk for CD. We here studied the impact of these polymorphisms on clinical characteristics in patients of the Swiss IBD Cohort Study (SIBDCS).

Methods: We included 981 Crohn's disease (CD) patients and 690 ulcerative colitis (UC) patients of the SIBDCS. We analyzed whether three CD-associated *NLRP3* polymorphisms have an impact on the clinical disease course in these patients.

Results: In CD patients presence of the major allele (G) of rs10733113 was associated with less surgeries and lower maximal CDAI and a similar trend was observed for rs55646866 and rs4353135. Presence of the major allele of all three SNPs was negatively correlated to maximal CDAI. In UC patients homozygous genotype for the major allele (CC) for rs55646866 was associated with a higher age at diagnosis and a higher MTWAI index. Homozygous genotype for the major allele of all three polymorphisms was associated with a higher number of ambulatory visits and longer hospital stays.

Conclusions: In CD patients presence of the major allele of all three polymorphisms was associated with markers of a less severe disease course, while in UC the homozygous genotype for all major alleles suggested a more severe disease activity.

Keywords: *NLRP3* inflammasome, Inflammatory bowel disease, Single nucleotide polymorphisms, Clinical characteristics

Background

Genetic variations in a predicted regulatory region of the NOD-like receptor pyrin domain containing 3 (*NLRP3*) gene locus have been associated with an increased risk to develop Crohn's disease (CD) [1]. CD risk has been linked to the major alleles of the *NLRP3* single nucleotide

*Correspondence: isabelle.frey@access.uzh.ch

⁵ Clinic for Gastroenterology and Hepatology, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland

Full list of author information is available at the end of the article



© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

polymorphisms (SNPs) rs10733113, rs4353135 and rs55646866; however, 64–85% of the healthy European or Canadian population carry the disease-associated alleles [1]. For an overview of the major allele frequency in different populations and for details on SNP location see Additional file 2: Table S1 and Additional file 1: Figure S1.

The protein NLRP3 belongs to the family of nucleotide binding oligomerization domain (NOD) and leucine rich repeat (LRR) containing receptors (NLRs). It has an important role in the innate immune response, namely sensing of pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs).

Upon activation, NLRP3 assembles with pro-caspase-1 and apoptosis-associated speck-like protein (ASC) to the NLRP3 inflammasome, resulting in autoproteolytic cleavage of pro-caspase-1 to active caspase-1 (CASP1). CASP1 is responsible for the conversion of pro-interleukin (IL)-1 β and pro-IL-18 to the active cytokines [2]. Inflammatory bowel disease (IBD) patients tend to have higher levels of these cytokines in the serum [3]. Elevated secretion of IL-1 β by lamina propria mononuclear cells isolated from inflamed IBD patients has been reported in several studies [4–6], and IL-1 β levels in colonic perfusion fluid correlated with disease activity [7] or risk of relapse within the next year [8]. Yet, experimental colitis in mice deficient in NLRP3 could not prove an unequivocal pro-inflammatory role of NLRP3. Depending on the animal model, environment and hygienic conditions, lack of NLRP3 either ameliorated or aggravated experimental colitis [9–12].

So far, little is known about the functional consequences of human *NLRP3* associated genetic variants. The major alleles of rs4353135 and rs10733113 have been shown to lead to lower NLRP3 mRNA expression in human peripheral blood mononuclear cells (PBMCs) [1, 13]. The major allele (GG) of rs6672995 was associated with lower IL-1 β secretion from stimulated PBMC [1], suggesting that the *NLRP3* polymorphisms that are associated with CD might lead to lower NLRP3-inflammasome activity. In line with the proposed pro-inflammatory role of the NLRP3 inflammasome during established IBD, polymorphisms that affect NLRP3 activity might also affect the clinical course of IBD patients, but such a relationship has not been addressed, so far.

We here studied for the first time whether the CD associated variants of the three SNPs (rs10733113, rs55646866, rs4353135) located in a regulatory region on chromosome 1q44 downstream of *NLRP3* affect the disease characteristics in IBD patients of the Swiss IBD cohort study (SIBDCS). In CD patients, the major allele of all three SNPs was negatively correlated to maximal CDAI, suggesting a less severe course of disease. In

contrast, in UC patients, presence of the major allele of rs55646866 was associated with a higher MTWAI while homozygous genotype for the major allele of all three polymorphisms was associated with a higher number of ambulatory visits. Our findings might lead to a better understanding of disease pathogenesis depending on *NLRP3* genotype and identify patients that might benefit from a NLRP3 targeted therapy.

Methods

Patient data

Data were obtained from the nationwide Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS). The SIBDCS is a prospective multicenter observational population-based study into which patients with IBD from all regions of Switzerland have been included since 2006. The goals and the methodology of the cohort have been described in detail previously [14, 15].

Study design

We included 1671 IBD patients (981 CD and 690 UC) that were enrolled in the SIBDCS at time of data acquisition and had been genotyped for the CD-associated single nucleotide polymorphisms (SNP) rs4353134, rs55646866 and rs10733113 within a regulatory region on chromosome 1q44 downstream of the *NLRP3* gene. Genotyping was performed as part of an analysis of the whole SIBDCS for selected SNPs that are currently known to be associated with IBD [16]. SNP Genotyping of SIBDCS samples was performed by MALDI-TOFF based analysis [17].

The aim of this study was to analyze whether the CD-associated risk variants for *NLRP3* rs4353134, rs55646866 and rs10733113 are associated with clinical parameters defining the severity/course of disease. For rs4353134, the T-allele is the major allele and the G-allele the minor allele. The major and minor alleles for rs55646866 are C/T and for rs10733113 G/A, respectively.

For analysis of clinical characteristics, we included gender, diagnosis, age at diagnosis, disease duration, complications, Crohn's disease activity index (CDAI) or modified Truelove and Witts activity index (MTWAI), reported flare (including possible cause of flare, flare management and hospitalization), fistula, abscess or anal fissure, stenosis, presence of extraintestinal manifestations, intestinal surgery and medication.

Statistical analysis

Clinical data were obtained from the data center of the SIBDCS at the University of Lausanne. These data and additional data obtained from a review of the patients' files were entered into a database (Access 2000; Microsoft Switzerland Ltd Liab. Co., Wallisellen, Switzerland).

Stata 14 software (StataCorp, 2015, College Station, TX) was used for the statistical analysis.

We analyzed for associations between clinical characteristics and genotype for rs4353135, rs55646866 and rs10733113 with Fisher’s exact test for discrete variables and with the Kruskal–Wallis test for continuous variables. To analyze for a cumulative risk of the three variants, we calculated a score based on the odds ratio for the risk allele as described by Villani et al. [1] for all patients (Score = $x * \log(\text{OR rs4353135 T-allele}) + y * \log(\text{OR rs55646866 C-allele}) + z * \log(\text{OR rs10733113 G-allele})$; x, y, z = number of major allele (0, 1 or 2)) resulting in a score between 0 and 2.58 for each patient. We performed linear regression analyses with this score as predictor and clinical parameters as response. Log(maxCDAI) was used for the analysis to reduce the impact of very high values.

For calculation of linkage disequilibrium (LD) we used the square of the correlation coefficient (r^2) between SNP pairs (R, genetics package, with the function LD()) <https://doi.org/10.1086/381000> [1].

Results

In this study, we investigated a total of 1671 IBD patients of the SIBDCS. 981 (58.7%) patients were classified as CD and 690 (41.3%) as UC patients.

Distribution of NLRP3 SNP genotypes in CD and UC patients

The distribution of the alleles for rs4353135, rs55646866 and rs10733113713 and the joint distribution of alleles

in CD and UC patients are presented in Tables 1 and 2. There were no significant differences in distribution of the genotypes between CD and UC patients. The major allele frequency in SIBDCS patients was 0.68 (T) for rs4353135, 0.89 (C) for rs55646866, and 0.74 (G) for rs10733113713, corresponding well with the major allele frequency in the European population. For an overview of the major allele frequencies in different populations see Additional file 2: Table S1. 422 (43.0%, CD) and 285 (41.3%, UC) patients are homozygous carriers for the major allele of all three SNPs. As CD and UC are distinct forms of IBD with diverging disease pathogenesis, we performed individual analyses for associations between NLRP3 SNPs and disease characteristics in CD and UC patients.

Presence of the major allele of all three SNPs is associated with lower maximal CDAI in CD patients

Presence of the major allele (G) of rs10733113 was associated with a significantly lower maximal CDAI value throughout follow-up (median CDAI = 63 in GG or AG vs. 76 in AA patients, $p = 0.011$, and we observed similar trends, yet not significant, for lower CDAI in carriers of the major alleles of rs4353135 and rs55646866 (Fig. 1A). The investigated SNPs have been reported to be linked due to close proximity of their loci [1] (they all lie within a 5321 bp region, for details see Additional file 1: Figure S1). In our cohort, however, the square of the correlation coefficient r^2 (Additional file 2: Table S2) was below 0.2, suggesting no relevant linkage of the studied SNPs. To analyze whether there is an additive effect

Table 1 Cross table of joint SNP distribution in CD patients

	rs10733113			Total
	GG	AG	AA	
rs4353135: TT	423 (90.8%)	1 (0.2%)	42 (9.0%)	466 (100%)
GT	252 (61.5%)	5 (1.2%)	153 (37.3%)	410 (100%)
GG	38 (36.2%)	1 (0.9%)	66 (62.9%)	105 (100%)
Total	713 (72.7%)	7 (0.7%)	261 (26.6%)	981 (100%)
rs55646866: CC	710 (90.4%)	3 (0.4%)	72 (9.2%)	785 (100%)
CT	3 (1.7%)	4 (2.3%)	166 (96.0%)	173 (100%)
TT	0 (0%)	0 (0%)	23 (100%)	23 (100%)
Total	713 (72.7%)	7 (0.7%)	261 (26.6%)	981 (100%)
	rs4353135			Total
	TT	GT	GG	
rs55646866: CC	465 (59.2%)	282 (35.9%)	38 (4.8%)	785 (100%)
CT	1 (0.6%)	128 (74.0%)	44 (25.4%)	173 (100%)
TT	0 (0%)	0 (0%)	23 (100%)	23 (100%)
Total	466 (47.5%)	410 (41.8%)	105 (10.7%)	981 (100%)

Table 2 Cross table of joint SNP distribution in UC patients

	rs10733113			Total
	GG	AG	AA	
rs4353135: TT	286 (90.2%)	3 (1.0%)	28 (8.8%)	317 (100%)
GT	196 (65.5%)	11 (3.7%)	92 (30.8%)	299 (100%)
GG	34 (45.9%)	0 (0%)	40 (54.1%)	74 (100%)
Total	516 (74.8%)	14 (2.0%)	160 (23.2%)	690 (100%)
rs55646866: CC	514 (90.7%)	4 (0.7%)	49 (8.6%)	567 (100%)
CT	2 (1.8%)	10 (9.0%)	99 (89.2%)	111 (100%)
TT	0 (0%)	0 (0%)	12 (100%)	12 (100%)
Total	516 (74.8%)	14 (2.0%)	160 (23.2%)	690 (100%)

	rs4353135			Total
	TT	GT	GG	
rs55646866: CC	316 (55.7%)	216 (38.1%)	35 (6.2%)	567 (100%)
CT	1 (0.9%)	82 (73.9%)	28 (25.2%)	111 (100%)
TT	0 (0%)	1 (8.3%)	11 (91.7%)	12 (100%)
Total	217 (45.9%)	299 (43.3%)	74 (10.7%)	690 (100%)

on the association between *NLRP3* SNPs and clinical parameters, we calculated a score for the cumulative genetic risk for carrying the major allele of all 3 polymorphisms. To calculate this score we added the log of the odds ratio (as identified by Villani et al. [1]) for the CD associated allele of the given SNP ($\log(1.78)=0.58$ for rs10733113, $\log(1.21)=0.19$ for rs4353135, $\log(1.69)=0.52$ for rs55646866) resulting in a score between 0 and 2.58 for each patient. In a linear regression model with this genetic score as predictor and $\log(\text{max. CDAI})$ as response we observed a significant negative association (Coefficient -0.097 ; 95% CI -0.182 to -0.011 ; $p=0.026$), indicating that carrying the CD-associated alleles for the 3 investigated *NLRP3* SNPs is associated with a less severe course of disease as evaluated by disease symptoms (Fig. 1B), and that there might be an additive effect of the three SNPs.

Carrying the major allele for rs55646844 is associated with longer disease duration in CD patients

Duration of disease, the time from diagnosis until last patient visit (i.e., the most recent patient visit, either at the regular yearly visit for the SIBDCS or the latest disease associated visit)

was significantly longer in patients carrying the major allele for rs55646866 (12.3 years for CC or CT vs. 6.1 years for TT, $p=0.013$). For rs4353135 a similar trend, yet not significant, could be observed (12.4 years for GT or TT vs. 9.9 years for GG, $p=0.090$) while there was no effect for rs10733113. Yet, age at diagnosis was not affected by the genotype for rs55646844 (Fig. 1C).

The major allele (GG) for rs10733113 is associated with fewer operations due to stenosis in CD patients

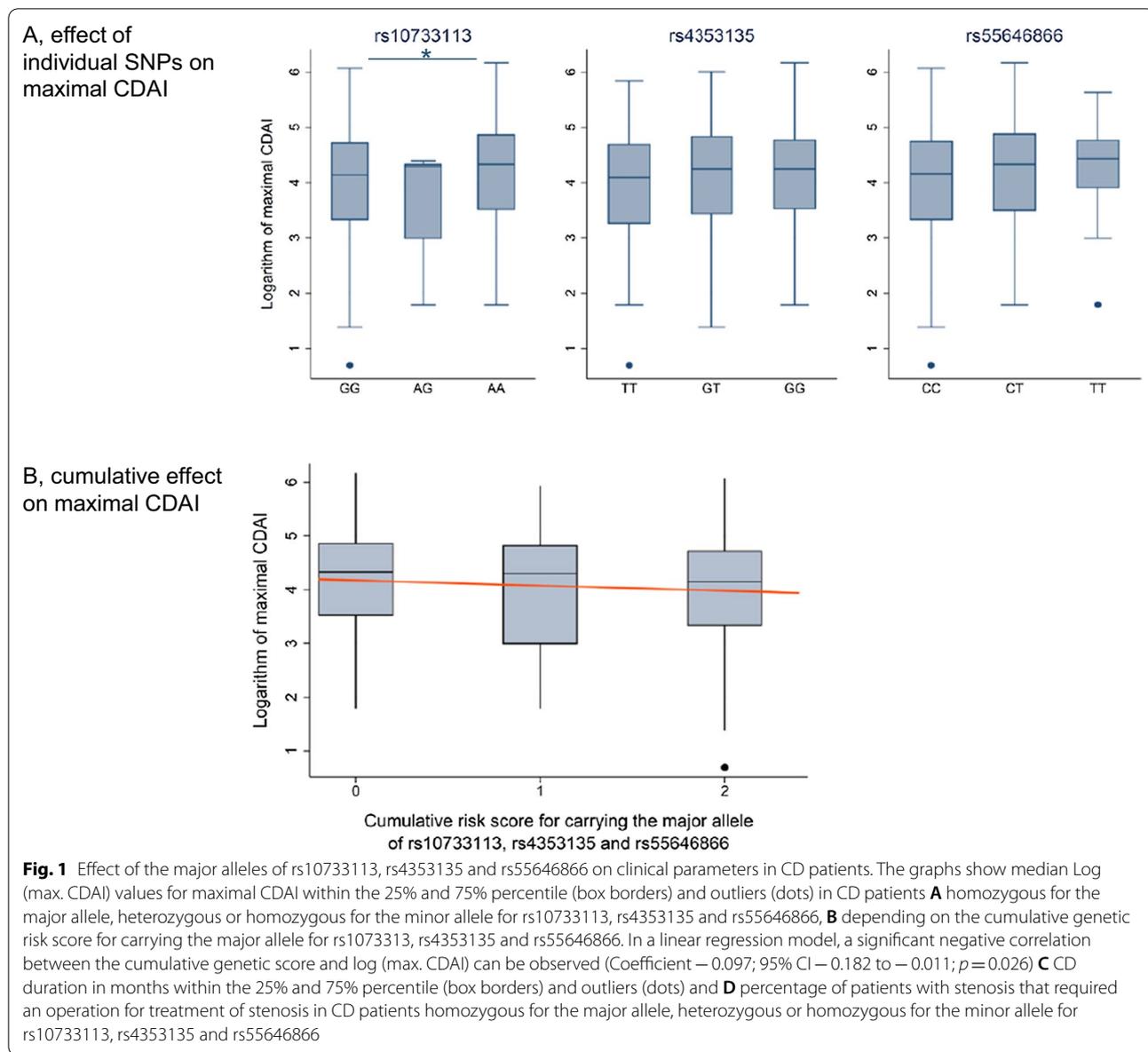
While the overall incidence of stenosis did not differ between genotypes, homozygous carriers of the major allele (GG) for rs10733113 had significantly less operations for treatment of stenosis (153 (49.4%) in GG, 0 (0%) in AG, 68 (58.1%) in AA, $p=0.045$). The genotype of the SNPs rs4353235 and rs55646866 was not associated to the frequency of operations needed for treatment of stenosis (Fig. 1D).

NLRP3 SNPs are not associated with further clinical disease characteristics in CD patients.

The major alleles of rs10733113, rs4353235 and rs55646866 were associated with a lower maximal CDAI value, suggesting a less severe course of disease in carriers of the major alleles. We analyzed whether individual clinical characteristics e.g. flare frequency, extraintestinal manifestations or medication are associated with carrying the major allele of the investigated polymorphisms. No further associations between genotype for rs10733113, rs4353235, rs55646866 and disease characteristics were observed in CD patients. Tables 3, 4 and 5 show the demographic distribution and clinical characteristics investigated for the different genotypes of rs10733113, rs4353235 and rs55646866.

Carrying the major alleles for rs10733113, rs4353235 and rs55646866 is associated with a higher number of ambulatory hospitalizations in UC patients

Additional ambulatory visits are strong indicators for occurrence of a moderate to severe flare in UC patients.



Homozygous genotype for the major allele of all three polymorphisms was associated with a higher number of ambulatory visits for flare management in UC patients (Fig. 2A). Despite their close proximity and earlier reports of linkage disequilibrium, the investigated polymorphisms showed low linkage disequilibrium in the SIBDCS (Additional file 2: Table S2) and we observed a significant association between the number of risk-associated alleles and the number of visits. We applied the same genetic score as calculated for the cumulative effect of all three SNPs on CDAI in CD patients for analysis of ambulatory flare management in UC patients. Based on a logistic regression, we find that this score is significantly

associated with the occurrence of at least one ambulatory flare measure (OR: 2.081 (95% CI 1.393–3.111; $p < 0.001$)) (Fig. 2B).

Homozygous genotype for the major allele (CC) of rs55646866 is associated with a higher age at disease onset and a higher MTWAI index

The MTWAI index characterizes the severity of disease as defined by symptoms in UC patients. Homozygous genotype (CC) for rs55646866 was associated with a higher MTWAI index in UC patients (mean MTWAI: 5 (CC), 5 (CT), 1.5 (TT), $p=0.009$). We further observed that homozygous genotype for the major allele of

Table 3 Demographic and clinical characteristics for the different genotypes of rs10733113 in CD patients

	rs10733113 GG	rs10733113 AG	rs10733113 AA	p value (Fisher or Kruskal- Wallis)
<i>Gender</i>				
Male (n = 488)	363 (50.9%)	4 (57.1%)	121 (46.4%)	0.390
Female (n = 493)	350 (49.1%)	3 (42.9%)	140 (53.6%)	
<i>Age at diagnosis [years]</i>				
Median, q25–q75,	24.7, 18.1–34.8,	21.3, 15.3–32.2,	23.4, 17.6–32.1,	0.164
Min–max	1.1–81.4	9.0–40.1	0.8–81.0	
<i>Disease duration [years]</i>				
Median, q25–q75,	12.1, 7.0–20.7,	7.5, 5.4–16.9,	12.7, 6.8–23.5,	0.462
Min–max	0.1–56.6	3.4–20.5	0.3–52.4	
<i>Complications*</i>				
No (n = 345)	259 (36.3%)	4 (57.1%)	82 (31.4%)	0.185
Yes (n = 636)	454 (63.7%)	3 (42.9%)	179 (68.6%)	
<i>Fistula, abscess or anal fissure*</i>				
No (n = 518)	376 (52.7%)	4 (57.1%)	138 (52.9%)	1.000
Yes (n = 463)	337 (47.3%)	3 (42.9%)	123 (47.1%)	
<i>Focus on the “yes”:</i>				
Perianal fistula	180 (53.4%)	3 (100%)	60 (48.8%)	0.214
Other fistula	129 (38.3%)	1 (33.3%)	53 (43.1%)	0.686
Fissure	102 (30.3%)	1 (33.3%)	37 (30.1%)	1.000
Abscess	184 (54.6%)	1 (33.3%)	68 (55.3%)	0.779
Surgery for fistula	178 (52.8%)	1 (33.3%)	67 (54.5%)	0.753
<i>Stenosis*</i>				
No (n = 551)	403 (56.5%)	4 (57.1%)	144 (55.2%)	0.916
Yes (n = 430)	310 (43.5%)	3 (42.9%)	117 (44.8%)	
<i>Focus on the “yes”:</i>				
Operation	153 (49.4%)	0 (0%)	68 (58.1%)	0.045
Dilation	117 (37.7%)	2 (66.7%)	50 (42.7%)	0.359
No intervention	170 (54.8%)	1 (33.3%)	65 (55.6%)	0.772
<i>CDAI**—maximal value throughout follow-up</i>				
Median, q25–q75,	63, 28–112,	73, 20–76,	76, 33–129,	0.024
Min–max	2–435	6–81	0–479	
<i>Reported flare*</i>				
No (n = 477)	355 (49.8%)	4 (57.1%)	118 (45.2%)	0.369
Yes (n = 504)	358 (50.2%)	3 (42.9%)	143 (54.8%)	
<i>Flare possibly or highly related to (focus on yes):</i>				
NSAIDs	9 (2.5%)	0 (0%)	2 (1.4%)	0.753
Antibiotics	8 (2.2%)	0 (0%)	1 (0.7%)	0.485
GI tract infection	54 (15.1%)	0 (0%)	30 (21.0%)	0.203
Other infection	23 (6.4%)	0 (0%)	14 (9.8%)	0.357
Treatment decr./disc	89 (24.9%)	1 (33.3%)	44 (30.8%)	0.338
Other medication	4 (1.1%)	0 (0%)	3 (2.1%)	0.437
<i>Flare management (focus on the yes):</i>				
Hospitalization	61 (17.0%)	1 (33.3%)	24 (16.8%)	0.598
Ambulatory	88 (24.6%)	0 (0%)	41 (28.7%)	0.468
Surgery	37 (10.3%)	0 (0%)	13 (9.1%)	0.813
Drug therapy	308 (86.0%)	3 (100%)	127 (88.8%)	0.650

Table 3 (continued)

	rs10733113 GG	rs10733113 AG	rs10733113 AA	<i>p</i> value (Fisher or Kruskal- Wallis)
<i>Focus on the hospitalizations:</i>				
Total days of hosp.	7, 2–17,	0, 0–0,	4.5, 1–13.5,	0.386
Median, q25–q75,	0–55	0–0	0–86	
Min–max				

rs55646866 was associated with a higher age at diagnosis (mean age at diagnosis: 28 (CC), 30 (CT), 18.3 (TT), $p=0.004$) (Fig. 2C).

***NLRP3* SNPs are not associated with further clinical disease characteristics in UC patients.**

The major alleles of rs10733113, rs4353235 and rs55646866 were associated with a higher number of ambulatory hospitalizations for flare management, suggesting a more severe course of disease in carriers of the major alleles. We therefore investigated whether individual clinical characteristics, e.g. flare frequency, extraintestinal manifestations or medication, that are usually affected during a more severe disease course, are associated with carrying the major allele for rs10733113, rs4353235 and rs55646866. No further associations between homozygous genotype for the major allele of rs10733113, rs4353235, rs55646866 and disease characteristics were observed in UC patients. Tables 6, 7 and 8 show the demographic distribution and clinical characteristics investigated for the different genotypes of rs10733113, rs4353235 and rs55646866.

Discussion

Polymorphisms in the regulatory region of *NLRP3* have been associated with an increased risk to develop CD, yet the impact of these variants on established IBD has not been clarified, so far. We here analyzed for possible associations between the SNPs rs10733113, rs4353135 and rs55646866 and the clinical characteristics of patients from the SIBDCS. Homozygous genotype for the major allele for rs10733113 was associated with a lower CDAI in CD patients. The same trend could be observed for rs4353135 and rs55646866. The SNPs rs4353135, rs55646866 and rs10733113 are in close proximity in the downstream region of *NLRP3*. Possible linkage disequilibrium between SNP pairs has been reported previously [1]: while *D'* values indicated linkage disequilibrium for all SNP pairs, r^2 showed very low linkage for 2 of the 3 SNP pairs. However, our calculations of r^2 values with SIBDCS data showed no indication of substantial linkage disequilibrium among the three studied SNPs. Furthermore, linear regression models showed log-additive allele

effects on maximal CDAI, suggesting independent effects of SNPs' alleles. Similarly, additive effects and a better diagnostic value of a genetic risk score in comparison to individual SNPs have recently been reported by Cleynen et al. [18]. As the CDAI describes the disease activity in CD patients, our findings suggest that the investigated CD-associated *NLRP3* variants are associated with a less severe course of disease. In addition, homozygous genotype for the major allele for rs10733113 was associated with fewer operations due to stenosis supporting our finding of an overall less severe course of disease in patients of this genotype.

The investigated polymorphisms in the regulatory region of *NLRP3* had been associated to CD, only, but not to UC [1]. Still, as similarly elevated IL-1 β levels have been reported for UC patients, suggesting that alterations in *NLRP3* inflammasome activity and IL-1 β levels might also affect clinical disease characteristics in UC patients, UC patients were also included in our analysis. In contrast to CD, UC patients homozygous for the major allele of the studied *NLRP3* polymorphisms seem to have a more severe course of disease. Homozygous genotype for all three polymorphisms was significantly associated with more days of hospitalization indicating most likely an acute flare. Again, the association with all three SNPs could not be explained by linkage disequilibrium, as we observed an additive risk for the genetic risk score for the number of ambulatory visits and did not find r^2 values indicative for linkage disequilibrium of these SNPs in the SIBDCS. In addition, homozygous genotype (GG) for the major allele of rs55646866 was associated with a higher MTWAI. As the MTWAI characterizes disease severity in UC patients, this finding further suggests a more severe course of disease in UC patients with GG genotype. In contrast, this genotype was associated with a higher age at diagnosis of disease. Higher age at diagnosis might suggest that the GG variant of rs55646866 is protective against development of UC, but we observed no further results that would support such a finding.

Further clinical characteristics, e.g. other parameters indicative of more severe disease like type of medication or need of treatment intensification, effects on CRP or calprotectin levels or nutrient deficiencies, were not

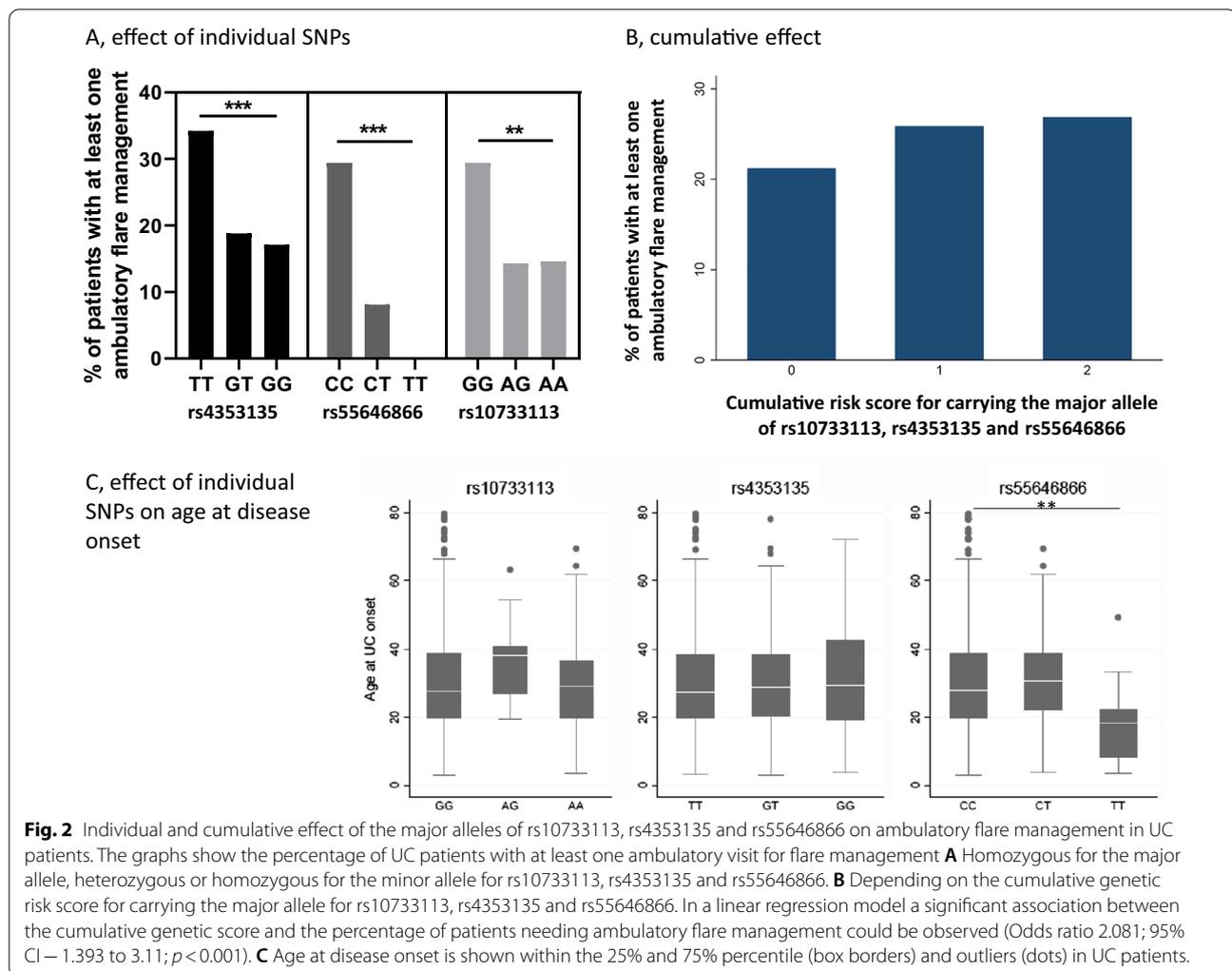
Table 4 Demographic and clinical characteristics for the different genotypes of rs4353135 in CD patients

	rs4353135 TT	rs4353135 GT	rs4353135 GG	p value (Fisher or Kruskal- Wallis)
<i>Gender</i>				
Male (n = 488)	234 (50.2%)	207 (50.5%)	47 (44.8%)	0.557
Female (n = 493)	232 (49.8%)	203 (49.5%)	58 (55.2%)	
<i>Age at diagnosis [years]</i>				
Median, q25–q75, Min–max	24.4, 18.2–34.7, 1.1–81.0	23.9, 17.9–32.5, 0.8–81.4	25.2, 17.4–35.1, 1.6–73.7	0.465
<i>Disease duration [years]</i>				
Median, q25–q75, Min–max	12.4, 7.4–21.0, 0.1–52.0	12.2, 7.2–21.2, 0.2–56.6	9.9, 5.7–19.4, 1.2–44.0	0.239
<i>Complications*</i>				
No (n = 345)	159 (34.1%)	147 (35.9%)	39 (37.1%)	0.765
Yes (n = 636)	307 (65.9%)	263 (64.1%)	66 (62.9%)	
<i>Fistula, abscess or anal fissure*</i>				
No (n = 518)	247 (53.0%)	221 (53.9%)	50 (47.6%)	0.513
Yes (n = 463)	219 (47.0%)	189 (46.1%)	55 (52.4%)	
<i>Focus on the “yes”:</i>				
Perianal fistula	117 (53.4%)	93 (49.2%)	33 (60.0%)	0.356
Other fistula	84 (36.4%)	84 (44.4%)	15 (27.3%)	0.066
Fissure	66 (30.1%)	52 (27.5%)	22 (40.0%)	0.207
Abscess	120 (54.8%)	107 (56.6%)	26 (47.3%)	0.474
<i>Surgery for fistula</i>	114 (52.1%)	102 (54.0%)	30 (54.5%)	0.913
<i>Stenosis*</i>				
No (n = 551)	256 (54.9%)	239 (58.3%)	56 (53.3%)	0.501
Yes (n = 430)	210 (45.1%)	171 (41.7%)	49 (46.7%)	
<i>Focus on the “yes”:</i>				
Operation	110 (52.4%)	83 (48.5%)	28 (57.1%)	0.541
Dilation	81 (38.6%)	72 (42.1%)	16 (32.7%)	0.469
No intervention	110 (52.4%)	98 (57.3%)	28 (57.1%)	0.595
<i>CDAI—maximal value throughout follow-up</i>				
Median, q25–q75, Min–max	60, 26–109, 0–345	70, 31–126, 4–405	70, 34–118, 6–479	0.071
<i>Reported flare*</i>				
No (n = 477)	240 (51.5%)	185 (45.1%)	52 (49.5%)	0.164
Yes (n = 504)	226 (48.5%)	225 (54.9%)	53 (50.5%)	
<i>Flare possibly or highly related to (focus on yes):</i>				
NSAIDs	5 (2.2%)	4 (1.8%)	2 (3.8%)	0.613
Antibiotics	5 (2.2%)	3 (1.3%)	1 (1.9%)	0.710
GI tract infection	35 (15.5%)	42 (18.7%)	7 (13.2%)	0.545
Other infection	15 (5.8%)	21 (9.3%)	3 (5.7%)	0.347
Treatment decr. / disc	50 (22.1%)	70 (31.1%)	14 (26.4%)	0.097
Other medication	1 (0.4%)	5 (2.2%)	1 (1.9%)	0.180
<i>Flare management (focus on the yes):</i>				
Hospitalization	35 (15.5%)	42 (18.7%)	9 (17.0%)	0.652
Ambulatory	59 (26.1%)	59 (26.2%)	11 (20.8%)	0.753
Surgery	18 (8.0%)	26 (11.6%)	6 (11.3%)	0.398
Drug therapy	198 (87.6%)	195 (86.7%)	45 (84.9%)	0.828
<i>Focus on the hospitalizations:</i>				
Total days of hosp. Median, q25–q75, Min–max	7, 0–12, 0–47	5.5, 2–17, 0–86	12, 3–20, 0–50	0.646

Table 5 Demographic and clinical characteristics for the different genotypes of rs55646866 in CD patients

	rs55646866 CC	rs55646866 CT	rs55646866 TT	p value (Fisher or Kruskal- Wallis)
<i>Gender</i>				
Male (n = 488)	394 (50.2%)	81 (46.8%)	13 (56.5%)	0.605
Female (n = 493)	391 (49.8%)	92 (53.2%)	10 (43.5%)	
<i>Age at diagnosis [years]</i>				
Median, q25–q75, Min–max	24.3, 17.9–34.4, 1.1–81.4	24.4, 18.5–33.0, 0.8–65.9	25.0, 18.7–31.5, 11.5–73.7	0.875
<i>Disease duration [years]</i>				
Median, q25–q75, Min–max	12.2, 7.0–20.9, 0.1–56.6	12.8, 7.6–23.5, 1.2–44.7	6.1, 4.5–13.1, 1.5–33.8	0.030
<i>Complications*</i>				
No (n = 345)	281 (35.8%)	56 (32.4%)	8 (34.8%)	0.727
Yes (n = 636)	504 (64.2%)	117 (67.6%)	15 (65.2%)	
<i>Fistula, abscess or anal fissure*</i>				
No (n = 518)	411 (52.4%)	94 (54.3%)	13 (56.5%)	0.836
Yes (n = 463)	374 (47.6%)	79 (45.7%)	10 (43.5%)	
<i>Focus on the “yes”:</i>				
Perianal fistula	199 (53.2%)	38 (48.1%)	6 (60.0%)	0.641
Other fistula	144 (38.5%)	36 (45.6%)	3 (30.0%)	0.439
Fissure	113 (30.2%)	24 (30.4%)	3 (30.0%)	1.000
Abscess	203 (54.3%)	44 (55.7%)	6 (60.0%)	0.930
Surgery	195 (52.1%)	46 (58.2%)	5 (50.0%)	0.626
<i>Stenosis*</i>				
No (n = 551)	446 (56.8%)	95 (54.9%)	10 (43.5%)	0.413
Yes (n = 430)	339 (43.2%)	78 (45.1%)	13 (56.5%)	
<i>Focus on the “yes”:</i>				
Operation	169 (49.9%)	44 (56.4%)	8 (61.5%)	0.460
Dilation	133 (39.2%)	33 (42.3%)	3 (23.1%)	0.434
No intervention	189 (55.8%)	40 (51.3%)	7 (53.8%)	0.766
<i>CDAI—maximal value throughout follow-up</i>				
Median, q25–q75, Min–max	64, 28–114, 0–435	76, 33–131, 6–479	64, 50–117, 6–280	0.108
<i>Reported flare*</i>				
No (n = 477)	387 (49.3%)	82 (47.4%)	8 (34.8%)	0.365
Yes (n = 504)	398 (50.7%)	91 (52.6%)	15 (65.2%)	
<i>Flare possibly or highly related to (focus on yes):</i>				
NSAIDs	8 (2.0%)	2 (2.2%)	1 (6.7%)	0.450
Antibiotics	8 (2.0%)	0 (0%)	1 (6.7%)	0.154
GI tract infection	63 (15.8%)	20 (22.0%)	1 (6.7%)	0.255
Other infection	25 (6.3%)	11 (12.1%)	1 (6.7%)	0.143
Treatment decr./disc	100 (25.1%)	29 (31.9%)	5 (33.3%)	0.319
Other medication	4 (1.0%)	3 (3.3%)	0 (0%)	0.292
<i>Flare management (focus on the yes):</i>				
Hospitalization	68 (17.1%)	16 (17.6%)	2 (13.3%)	0.967
Ambulatory	96 (24.1%)	31 (34.1%)	2 (13.3%)	0.090
Surgery	39 (9.8%)	11 (12.1%)	0 (0%)	0.443
Drug therapy	344 (86.4%)	81 (89.0%)	13 (86.7%)	0.776
<i>Focus on the hospitalizations:</i>				
Total days of hosp. Median, q25–q75, Min–max	7.5, 2–16, 0–55	3.5, 0–17.5, 0–86	4, 3–5, 3–5	0.553

Bold indicates a significant p-value (p < 0.05)



affected by *NLRP3* variants, neither in CD nor in UC patients.

Genetic determination of high interindividual variation of in vitro IL-1 β secretion and association with disease course in CD and UC patients has been previously reported [19, 20]. So far, little is known about the functional consequences of the investigated SNPs in the regulatory region of *NLRP3*. *NLRP3* mRNA expression was lower in PBMCs isolated from carriers of the major alleles of rs4353135 and rs10733113 in the CD-cohorts studied by Villani et al. and in patients with myocardial infarction and healthy controls [1, 13]. IL-1 β secretion from isolated PBMCs was lower in patients with homozygous genotype for the major allele (GG) of SNP rs6672995 [1] and the same trend could be observed for homozygous genotype for the major alleles of rs55646866 and rs10733113. Homozygous genotype for the minor allele of rs4353135 was further associated with a higher risk for oligoarticular and polyarticular juvenile idiopathic

arthritis and higher levels of inflammatory markers in a Taiwanese population [21], while presence of the G-allele of rs10733113 was associated with early onset disease in psoriatic arthritis [22].

These findings suggest that the *NLRP3* polymorphisms that are associated with CD might lead to lower *NLRP3*-inflammasome activity and lower IL-1 β secretion. In line with the proposed pro-inflammatory role for *NLRP3* and IL-1 β , such lower *NLRP3* activity and IL-1 β secretion, might indeed be causally related to a less severe disease course resulting in a lower CDAI in CD patients homozygous for the major allele for these SNPs. As we observed a cumulative effect of the investigated *NLRP3* variants on CDAI it would be of particular interest to investigate whether being homozygous for the major allele for more than one of the investigated SNPs has a cumulative effect on IL-1 β secretion, thus that patients homozygous for all three major alleles would have the lowest IL-1 β levels. Furthermore, polymorphisms in the *IL1B* gene have

Table 6 Demographic and clinical characteristics for the different genotypes of rs10733113 in UC patients

	rs10733113 GG	rs10733113 AG	rs10733113 AA	p value (Fisher or Kruskal- Wallis)
<i>Gender</i>				
Male (n = 369)	276 (53.5%)	10 (71.4%)	83 (51.9%)	0.392
Female (n = 321)	240 (46.5%)	4 (28.6%)	77 (48.1%)	
<i>Age at diagnosis [years]</i>				
Median, q25–q75, Min–max	27.7, 19.8–38.7, 2.9–79.6	38.0, 27.1–40.8, 19.5–63.2	29.2, 19.7–36.7, 3.4–69.4	0.099
<i>Disease duration [years]</i>				
Median, q25–q75, Min–max	11.1, 6.7–18.4, 0.2–49.5	10.7, 7.8–17.8, 2.9–26.3	11.7, 7.3–18.8, 0.5–40.1	0.619
<i>Complications*</i>				
No (n = 286)	225 (43.6%)	6 (42.9%)	55 (34.4%)	0.122
Yes (n = 404)	291 (56.4%)	8 (57.1%)	105 (65.6%)	
<i>MTWAI–maximal value throughout follow-up</i>				
Median, q25–q75, Min–max	4, 2–8.5, 0–19	7.5, 3–9, 0–13	5, 2–9, 0–17	0.704
<i>Reported flare*</i>				
No (n = 284)	213 (41.3%)	7 (50.0%)	64 (40.0%)	0.753
Yes (n = 406)	303 (58.7%)	7 (50.0%)	96 (60.0%)	
<i>Flare possibly or highly related to (focus on yes):</i>				
NSAIDs	19 (6.3%)	0 (0%)	4 (4.2%)	0.746
Antibiotics	6 (2.0%)	1 (14.3%)	1 (1.0%)	0.150
GI tract infection	51 (16.8%)	1 (14.3%)	22 (22.9%)	0.347
Other infection	29 (9.6%)	0 (0%)	10 (10.4%)	0.924
Treatment decr. / disc	74 (24.4%)	2 (28.6%)	22 (22.9%)	0.864
Other medication	9 (3.0%)	0 (0%)	5 (5.2%)	0.484
<i>Flare management (focus on the yes):</i>				
Hospitalization	39 (12.9%)	0 (0%)	7 (7.3%)	0.278
Ambulatory	89 (29.4%)	1 (14.3%)	14 (14.6%)	0.008
Surgery	16 (5.3%)	0 (0%)	3 (3.1%)	0.702
Drug therapy	271 (89.4%)	7 (100%)	90 (93.8%)	0.367
<i>Focus on the hospitalizations:</i>				
Total days of hosp. Median, q25–q75, Min–max	4, 0–16, 0–90	–	0, 0–3, 0–12	0.063

Bold indicates a significant p-value ($p < 0.05$)

been reported to affect course and severity of IBD [23] and nucleotide-binding domain and leucine-rich repeat caspase recruitment domain 4 (NLRC4) and NLRP3 can be recruited to the same macromolecular inflammatory complex [24]. Although no associations between *NLRC4* or *CASP1* polymorphisms and IBD have been reported so far, it would be very interesting to investigate whether IL-1 β levels are affected by cumulative effects of polymorphisms in *IL1B* itself and further genes involved in IL-1 β processing. Unfortunately, such an analysis is beyond the scope of our study.

CD and UC are distinct clinical entities with differences in disease location, histology and only partly shared genetic susceptibility [25]. Still, the diverging effect of CD-associated *NLRP3* polymorphisms on disease characteristics in CD patients and UC patients is

striking, as similarly elevated secretion levels of IL-1 β have been observed in CD and UC patients [4–6, 8, 26]. The diverging role of *NLRP3* polymorphisms on clinical parameters in CD and UC patients remains unexplained and the role of NLRP3 and IL-1 β in IBD is still elusive, as reviewed in detail recently [27]. Although consistently elevated levels of IL-1 β secretion have been reported for both CD and UC patients, IL-1 β does not seem to play a decisive role in the inflammatory process of IBD patients, as only patients with IL-10R deficiency responded to IL-1R-antagonist treatment [27]. Data from murine models of experimental colitis with animals lacking NLRP3 show diverging effects depending on the model, experimental conditions and environment. Both, aggravated as well as less severe course of disease have been reported for *Nlrp3*^{-/-} mice during acute DSS and TNBS colitis [9,

Table 7 Demographic and clinical characteristics for the different genotypes of rs4353135 in UC patients

	rs4353135 TT	rs4353135 GT	rs4353135 GG	p value (Fisher or Kruskal- Wallis)
<i>Gender</i>				
Male (n = 369)	169 (53.3%)	165 (55.2%)	35 (47.5%)	0.474
Female (n = 321)	148 (46.7%)	134 (44.8%)	39 (52.7%)	
<i>Age at diagnosis [years]</i>				
Median, q25–q75, Min–max	27.4, 19.7–38.4, 3.2–79.6	28.7, 20.4–38.4, 2.9–78.1	29.3, 19.2–42.6, 3.9–72.1	0.628
<i>Disease duration [years]</i>				
Median, q25–q75, Min–max	10.9, 6.9–18.4, 0.3–49.5	11.5, 6.9–17.6, 0.2–48.7	13.1, 6.9–22.5, 1.0–45.3	0.580
<i>Complications*</i>				
No (n = 286)	135 (42.6%)	125 (41.8%)	26 (35.1%)	0.507
Yes (n = 404)	182 (57.4%)	174 (58.2%)	48 (64.9%)	
<i>MTWAI**—maximal value throughout follow-up</i>				
Median, q25–q75, Min–max	4, 2–9, 0–19	5, 2–9, 0–18	4, 2–7, 0–14	0.129
<i>Reported flare*</i>				
No (n = 284)	121 (38.2%)	130 (43.5%)	33 (44.6%)	0.329
Yes (n = 406)	196 (61.8%)	169 (56.5%)	41 (55.4%)	
<i>Flare possibly or highly related to (focus on yes):</i>				
NSAIDs	11 (5.6%)	11 (6.5%)	1 (2.4%)	0.705
Antibiotics	4 (2.0%)	4 (2.4%)	0 (0%)	1.000
GI tract infection	32 (16.3%)	34 (20.1%)	8 (19.5%)	0.632
Other infection	23 (11.7%)	11 (6.5%)	5 (12.2%)	0.168
Treatment dect./disc	50 (25.5%)	41 (24.3%)	7 (17.1%)	0.543
Other medication	4 (2.0%)	9 (5.3%)	1 (2.4%)	0.219
<i>Flare management (focus on the yes):</i>				
Hospitalization	24 (12.2%)	20 (11.8%)	2 (4.9%)	0.443
Ambulatory	67 (34.2%)	30 (17.8%)	7 (17.1%)	0.001
Surgery	8 (4.1%)	10 (5.9%)	1 (2.4%)	0.692
Drug therapy	178 (90.8%)	150 (88.8%)	40 (97.6%)	0.248
<i>Focus on the hospitalizations:</i>				
Total days of hosp. Median, q25–q75, Min–max	6.5, 2.5–18.5, 0–90	0, 0–7, 0–41	3, 0–6, 0–6	0.038

Bold indicates a significant p-value ($p < 0.05$)

10, 12, 28, 29]. Itani et al. describe a more severe course of disease of *Nlrp3*^{-/-} mice during oxazolone colitis [11], while Mak'Anyengo et al. observed reduced colitis in *Nlrp3*^{-/-} with the T-cell transfer colitis model [30]. These findings may be explained by the different roles of IL- β during intestinal inflammation: Mak'Anyengo et al. showed NLRP3 dependent IL-1 β promoted Th17 differentiation and GM-CSF production of T-cells acting pro-inflammatory and resulting in aggravated colitis. Bersudsky et al. on the other hand, demonstrated that not IL-1 β but IL-1 α is the decisive pro-inflammatory mediator in the DSS colitis model, whereas IL-1 β supports epithelial cell proliferation and restoration of colon barrier [31].

Data from patients or animals with NLRP3 hyperactivity provide a similar controversial picture for the

role of NLRP3 in colitis. Both aggravation as well as amelioration of colitis have been shown in patients and mice with various reasons of NLRP3 hyperactivation. Lack of negative regulation by IL-10 leads to NLRP3 overactivation in *Il10*^{-/-} mice that develop spontaneous colitis [32, 33]. In this model the pro-inflammatory role of IL-1 β seems to be non-redundant as treatment with a CASP1 or a NLRP3 inhibitor ameliorated established disease and prevented or delayed development of spontaneous colitis. Similarly, lack of negative regulation of NLRP3 by mutated CARD8 was found to be responsible for development of CD in three related patients, as disease was responsive to IL-1 β inhibition [34].

On the contrary, situations with protective effects of NLRP3 hyperactivation have been observed in mice carrying an activating, autoimmune-associated mutation of

Table 8 Demographic and clinical characteristics for the different genotypes of rs55646866 in UC patients

	rs55646866 CC	rs55646866 CT	rs55646866 TT	p value (Fisher or Kruskal- Wallis)
<i>Gender</i>				
Male (n = 369)	302 (53.3%)	63 (56.8%)	4 (33.3%)	0.302
Female (n = 321)	265 (46.7%)	48 (43.2%)	8 (66.7%)	
<i>Age at diagnosis [years]</i>				
Median, q25–q75, Min–max	28.0, 19.9–38.7, 2.9–79.6	30.7, 22.1–38.8, 3.9–69.4	18.3, 8.5–22.1, 3.4–49.2	0.004
<i>Disease duration [years]</i>				
Median, q25–q75, Min–max	11.1, 6.7–18.1, 0.2–49.5	13.2, 7.8–20.0, 0.5–38.3	10.2, 7.0–14.2, 4.6–40.1	0.192
<i>Complications*</i>				
No (n = 286)	244 (43.0%)	39 (35.1%)	3 (25.0%)	0.160
Yes (n = 404)	323 (57.0%)	72 (64.9%)	9 (75.0%)	
<i>MTWAI—maximal value throughout follow-up</i>				
Median, q25–q75, Min–max	5, 2–9, 0–19	5, 2–9, 0–17	1.5, 0–2.5, 0–14	0.009
<i>Reported flare*</i>				
No (n = 284)	230 (40.6%)	49 (44.1%)	5 (41.7%)	0.777
Yes (n = 406)	337 (59.4%)	62 (55.9%)	7 (58.3%)	
<i>Flare possibly or highly related to (focus on yes):</i>				
NSAIDs	20 (5.9%)	3 (4.8%)	0 (0%)	1.000
Antibiotics	6 (1.8%)	2 (3.2%)	0 (0%)	0.444
GI tract infection	57 (16.9%)	15 (24.2%)	2 (28.6%)	0.249
Other infection	32 (9.5%)	5 (8.1%)	2 (28.6%)	0.223
Treatment dect./disc	86 (25.5%)	10 (16.1%)	2 (28.6%)	0.232
Other medication	10 (3.0%)	4 (6.5%)	0 (0%)	0.413
<i>Flare management (focus on the yes):</i>				
Hospitalization	42 (12.5%)	4 (6.5%)	0 (0%)	0.375
Ambulatory	99 (29.4%)	5 (8.1%)	0 (0%)	< 0.001
Surgery	17 (5.0%)	2 (3.2%)	0 (0%)	0.822
Drug therapy	302 (89.6%)	59 (95.2%)	7 (100%)	0.376
<i>Focus on the hospitalizations:</i>				
Total days of hosp. Median, q25–q75, Min–max	4, 0–14, 0–90	0, 0–6, 0–12	–	0.225

Bold indicates a significant p-value ($p < 0.05$)

Ptpn22 [29]. As NLRP3 is activated by PTPN22-dephosphorylation, the *Ptpn*^{619W} variant leads to increased NLRP3 activation and IL-1 β secretion. Mice carrying this mutation were protected from DSS colitis. Likewise, mice carrying the same activating mutation (*Nlrp3*^{R258W}) that is responsible for increased NLRP3 activation and IL-1 β levels in patients with cryoporin associated periodic syndrome (CAPS) were resistant to DSS colitis and T cell transfer colitis [35]. In these animals NLRP3 hyperactivation led to remodelling of the gut microbiota and increased production of regulatory T cells and antimicrobial peptides.

As gut microbiota alterations have been reported for animal models of both lack [10] or hyperactivation [35] of NLRP3, the question arises, whether NLRP3

variant induced modulation of the gut microbiota might be involved in the effect on IBD disease course. Yet, Yilmaz et al. [36] performed an extensive analysis of microbiota disturbances in the SIBDCS, but did not report effects of polymorphisms and we are not aware of any other study investigating the impact of NLRP3 SNPs on the gut microbiota.

So far, it is not clear whether polymorphisms that affect IBD risk also contribute to disease course and severity. While O'Donnell et al. [37] report association of 8 SNPs with time to-abdominal surgery, Lee et al. [38] could not identify any associations of disease susceptibility loci with disease prognosis, but identified four loci with significant association to the course of CD without any association to disease susceptibility. In addition, TLR

SNPs that do not affect disease susceptibility, were found to be associated with extensive colonic disease in both CD and UC patients [39], and polymorphisms associated with smoking behavior could predict the number of surgeries and the time to first intestinal surgery in smoking Crohn's disease patients [40].

Studies addressing the association of genetic loci to disease prognosis included a smaller number of patients in contrast to large genome wide association studies addressing IBD risk. We here studied 981 CD and 690 UC patients leading to significant associations, not robust to Bonferroni correction. Therefore, the identified associations between *NLRP3* polymorphisms and IBD prognosis need to be confirmed in additional cohorts.

Based on the currently available (sparse) data, that homozygous genotype for the major allele of CD associated *NLRP3* variants leads to lower *NLRP3* mRNA levels and activity resulting in lower IL-1 β secretion, our findings suggest that during established CD the pro-inflammatory role of IL-1 β might dominate, thus that patients homozygous for the major allele of these SNPs might profit from a less severe course of disease. In UC patients on the other hand, our findings support a protective role of *NLRP3* such that homozygous genotype for the major alleles of the investigated *NLRP3* polymorphisms might favour a more severe course of disease.

The fact, that the major alleles of *NLRP3* polymorphisms are the CD associated alleles implies that a considerable number of CD patients—in our study 43%—might profit from the beneficial effect on disease severity. On the other hand, in CD patients homozygous for the minor allele, potentially resulting in a more active *NLRP3* inflammasome, newly available *NLRP3* inhibitors, e.g. MCC950 or Cy-09 [41, 42] might present a novel treatment option. In contrast, based on our findings that the major alleles of CD-associated *NLRP3* variants were associated with more days of hospitalization in UC patients, treatment with *NLRP3* inhibitors might be detrimental in UC patients. Nevertheless, a detailed analysis of the effects of *NLRP3* variants on *NLRP3*-inflammasome activity and on IL-1 β cytokine levels in IBD patients is necessary, before considering *NLRP3* variant data for treatment decisions.

Conclusions

In summary, we here show that SNPs in the regulatory region of the *NLRP3* gene have diverging effects on the course of disease in CD and UC patients. Our findings should be taken into account, if treatment with novel *NLRP3* inhibitors is considered. Yet, further confirmation of the impact of *NLRP3* polymorphisms on IBD disease course in additional and/or larger cohorts and

a more detailed analysis of the consequences of *NLRP3* variants is necessary.

Abbreviations

NLRP3: NOD-like receptor pyrin domain containing 3; SNP: Single nucleotide polymorphism; SIBDCS: Swiss IBD cohort study; CD: Crohn's disease; UC: Ulcerative colitis; PAMP: Pathogen associated molecular pattern; DAMP: Danger associated molecular pattern; NOD: Nucleotide binding oligomerization domain; LRR: Leucine rich repeat; NLR: NOD-like receptor; CDAl: Crohn's disease activity index; MTWAl: Truelove and Witts activity index; NLR4: Nucleotide-binding domain and leucine-rich repeat caspase recruitment domain 4.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12876-021-01880-9>.

Additional file 1. Figure S1.

Additional file 2. Table S1.

Acknowledgements

The authors thank all the patients for their collaboration and the members of the Swiss Inflammatory Bowel Disease Cohort Study for their contribution. Members of the Swiss IBD Cohort Study Group: Karim Abdelrahman, Gentiana Ademi, Patrick Aepli, Amman Thomas, Claudia Anderegg, Anca-Teodora Antonino, Eva Archanioti, Eviano Arrigoni, Diana Bakker de Jong, Bruno Balsiger, Polat Bastürk, Peter Bauerfeind, Andrea Becocci, Dominique Belli, José M. Bengoa, Luc Biedermann, Janek Binek, Mirjam Blattmann, Stephan Boehm, Tujana Boldanova, Jan Borovicka, Christian P. Braegger, Stephan Brand, Lukas Brügger, Simon Brunner, Patrick Bühler, Bernard Burnand, Sabine Burk, Emanuel Burri, Sophie Buyse, Dahlia-Thao Cao, Ove Carstens, Dominique H. Criblez, Sophie Cunningham, Fabrizia D'Angelo, Philippe de Saussure, Lukas Degen, Joakim Delarive, Christopher Doerig, Barbara Dora, Susan Drerup, Mara Egger, Ali El-Wafa, Matthias Engelmann, Jessica Ezri, Christian Felley, Markus Fliegner, Nicolas Fournier, Montserrat Fraga, Yannick Franc, Pascal Frei, Remus Frei, Michael Fried, Florian Froehlich, Raoul Ivano Furlano, Luca Garzoni, Martin Geyer, Laurent Girard, Marc Girardin, Delphine Golay, Ignaz Good, Ulrike Graf Bigler, Beat Gysi, Johannes Haarer, Marcel Halama, Janine Haldemann, Pius Heer, Benjamin Heimgartner, Beat Helbling, Peter Hengstler, Denise Herzog, Cyrill Hess, Roxane Hessler, Klaas Heyland, Thomas Hinterleitner, Claudia Hirschi, Petr Hruz, Pascal Juillerat, Carolina Khalid-de Bakker, Stephan Kayser, Céline Keller, Christina Knellwolf (-Grieger), Christoph Knoblauch, Henrik Köhler, Rebekka Koller, Claudia Krieger(-Grübel), Patrizia Künzler, Rachel Kusche, Frank Serge Lehmann, Andrew Macpherson, Michel H. Maillard, Michael Manz, Astrid Marot, Rémy Meier, Christa Meyenberger, Pamela Meyer, Pierre Michetti, Benjamin Misselwitz, Patrick Mosler, Christian Mottet, Christoph Müller, Beat Müllhaupt, Leilla Musso, Michaela Neagu, Cristina Nichita, Jan Niess, Andreas Nydegger, Nicole Obialo, Diana Ollo, Cassandra Oropesa, Ulrich Peter, Daniel Peternac, Laetitia Marie Petit, Valérie Pittet, Daniel Pohl, Marc Porzner, Claudia Preissler, Nadia Raschle, Ronald Rentsch, Alexandre Restellini, Sophie Restellini, Jean-Pierre Richterich, Frederic Ris, Branislav Risti, Marc Alain Ritz, Gerhard Rogler, Nina Röhrich, Jean-Benoît Rossel, Vanessa Rueger, Monica Rusticeanu, Markus Sagmeister, Gaby Saner, Bernhard Sauter, Mikael Sawatzki, Michael Scharl, Martin Schelling, Susanne Schibli, Hugo Schlauri, Dominique Schluckebier, Daniela Schmid, Sybille Schmid (-Uebelhart), Jean-François Schnegg, Alain Schoepfer, Vivianne Seematter, Frank Seibold, Mariam Seirafi, Gian-Marco Semadeni, Arne Senning, Christiane Sokolik, Joachim Sommer, Johannes Spalinger, Holger Spangenberger, Philippe Stadler, Peter Staub, Dominic Staudenmann, Volker Stenz, Michael Steuerwald, Alex Straumann, Bruno Strebel, Andreas Stulz, Michael Sulz, Aurora Tatu, Michela Tempia-Caliera, Joël Thorens, Kaspar Truninger, Radu Tutuian, Patrick Urfer, Stephan Vavricka, Francesco Viani, Jürg Vögtlin, Roland Von Känel, Dominique Vouillamoz, Rachel Vulliamy, Paul Wiesel, Reiner Wiest, Stefanie Wöhrle, Samuel Zamora, Silvan Zander, Tina Wylie, Jonas Zeitz, Dorothee Zimmermann. Head of the Swiss IBD study group is Prof. Gerhard Rogler; Division

of Gastroenterology and Hepatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland; e-mail: Gerhard.rogler@usz.ch

Authors' contributions

BM, MH, GR, MS and IFW: concept and design of the study; PY, JBR, SBUJ, YF, LB, BM, IFW: acquisition and analysis of the data; JBR, SBUJ, YF and IFW: statistical analysis and visualization, PY, JBR and IFW drafting the article; JBR, SBUJ, YF, LB, BM, MH, GR, MS and IFW interpretation of the data and critical review. All authors read and approved the final manuscript.

Funding

This research was supported by a research grant from the Swiss National Science Foundation to GR for the Swiss IBD Cohort (Grant No. 3347CO-108792). Funding enabled data acquisition and the genetic analysis of the SIBDC. The analysis at hand was performed by the authors without external funding. The funder did not influence the design of the study, the collection, analysis, and interpretation of data, nor the writing of the manuscript.

Availability of data and materials

Data may be made available upon request due to ethical restrictions imposed by the Swiss IBD cohort study group which collected this data. Interested researchers may request access to the collected data from this study in the same manner as the authors did. Data is available upon ethical approval and a request to the Head of the cohort, Dr. Gerhard Rogler, at gerhard.rogler@usz.ch. Data access requests may also be made to the Swiss IBD Cohort at sibdcs-submission@chuv.ch or <http://ibdcohort.ch/index.php/informationen-fuer-forscher.html>.

Declarations

Ethics approval and consent to participate

The SIBDCS has been approved by the respective local ethics committees in Switzerland (BASEC-No. 2018-02068, approved on 09.03.2020 by the Cantonal Ethics Committee of the Canton Zurich, Switzerland) conforming to the ethical guidelines of the 1975 Declaration of Helsinki. All patients signed an informed consent and confirmed their participation in the cohort study at the time of enrolment and gave informed consent for data collection and analysis for research purposes. The current substudy has been evaluated and approved by the scientific board of SIBDCS.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests relevant to this study.

Author details

¹Department of Gastroenterology and Hepatology, University Hospital and University of Zurich, Zurich, Switzerland. ²Center for Primary Care and Public Health (Unisanté), University of Lausanne, Lausanne, Switzerland. ³Department of Visceral Surgery and Medicine, Inselspital Bern and Berne University, Berne, Switzerland. ⁴Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland. ⁵Clinic for Gastroenterology and Hepatology, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland. ⁶Present Address: Institute of Medical Microbiology, University of Zurich, Gloriastrasse 28/30, 8006 Zurich, Switzerland.

Received: 17 January 2021 Accepted: 10 July 2021

Published online: 03 August 2021

References

- Villani AC, Lemire M, Fortin G, et al. Common variants in the NLRP3 region contribute to Crohn's disease susceptibility. *Nat Genet*. 2009;41:71–6.
- Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med*. 2015;21:677–87.
- Sartor RB. Cytokines in intestinal inflammation: pathophysiological and clinical considerations. *Gastroenterology*. 1994;106:533–9.
- Grottrup-Wolfers E, Moeller J, Karbach U, et al. Elevated cell-associated levels of interleukin 1beta and interleukin 6 in inflamed mucosa of inflammatory bowel disease. *Eur J Clin Invest*. 1996;26:115–22.
- Mahida YR, Wu K, Jewell DP. Enhanced production of interleukin 1-beta by mononuclear cells isolated from mucosa with active ulcerative colitis of Crohn's disease. *Gut*. 1989;30:835–8.
- Reinecker HC, Steffen M, Witthoef T, et al. Enhanced secretion of tumour necrosis factor-alpha, IL-6, and IL-1 beta by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. *Clin Exp Immunol*. 1993;94:174–81.
- Guimbaud R, Bertrand V, Chauvelot-Moachon L, et al. Network of inflammatory cytokines and correlation with disease activity in ulcerative colitis. *Am J Gastroenterol*. 1998;93:2397–404.
- Schreiber S, Nikolaus S, Hampe J, et al. Tumour necrosis factor alpha and interleukin 1beta in relapse of Crohn's disease. *Lancet*. 1999;353:459–61.
- Bauer C, Duewell P, Mayer C, et al. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. *Gut*. 2010;59:1192–9.
- Hirota SA, Ng J, Lueng A, et al. NLRP3 inflammasome plays a key role in the regulation of intestinal homeostasis. *Inflamm Bowel Dis*. 2011;17:1359–72.
- Itani S, Watanabe T, Nadatani Y, et al. NLRP3 inflammasome has a protective effect against oxazolone-induced colitis: a possible role in ulcerative colitis. *Sci Rep*. 2016;6:39075.
- Zaki MH, Boyd KL, Vogel P, et al. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity*. 2010;32:379–91.
- Paramel Varghese G, Folkersen L, Strawbridge RJ, et al. NLRP3 inflammasome expression and activation in human atherosclerosis. *J Am Heart Assoc*. 2016;5:e003031.
- Pittet V, Juillerat P, Mottet C, et al. Cohort profile: the Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS). *Int J Epidemiol*. 2009;38:922–31.
- Pittet V, Michetti P, Mueller C, et al. Cohort profile update: the Swiss inflammatory bowel disease cohort study (SIBDCS). *Int J Epidemiol*. 2019;48:385–386f.
- Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*. 2012;491:119–24.
- Storm N, Darnhofer-Patel B, van den Boom D, et al. MALDI-TOF mass spectrometry-based SNP genotyping. *Methods Mol Biol*. 2003;212:241–62.
- Cleynen I, Boucher G, Jostins L, et al. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. *Lancet*. 2016;387:156–67.
- Endres S, Cannon JG, Ghorbani R, et al. In vitro production of IL 1 beta, IL 1 alpha, TNF and IL2 in healthy subjects: distribution, effect of cyclooxygenase inhibition and evidence of independent gene regulation. *Eur J Immunol*. 1989;19:2327–33.
- Nemetz A, Nosti-Escanilla MP, Molnar T, et al. IL1B gene polymorphisms influence the course and severity of inflammatory bowel disease. *Immunogenetics*. 1999;49:527–31.
- Yang CA, Huang ST, Chiang BL. Association of NLRP3 and CARD8 genetic polymorphisms with juvenile idiopathic arthritis in a Taiwanese population. *Scand J Rheumatol*. 2014;43:146–52.
- Juneblad K, Kastbom A, Johansson L, et al. Association between inflammasome-related polymorphisms and psoriatic arthritis. *Scand J Rheumatol*. 2021;50:206–12.
- Nemetz A, Kope A, Molnar T, et al. Significant differences in the interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms in a Hungarian population with inflammatory bowel disease. *Scand J Gastroenterol*. 1999;34:175–9.
- Man SM, Hopkins LJ, Nugent E, et al. Inflammasome activation causes dual recruitment of NLRC4 and NLRP3 to the same macromolecular complex. *Proc Natl Acad Sci U S A*. 2014;111:7403–8.
- Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature*. 2011;474:307–17.
- Lazaridis LD, Pistiki A, Giamarellos-Bourboulis EJ, et al. Activation of NLRP3 inflammasome in inflammatory bowel disease: differences between Crohn's disease and ulcerative colitis. *Dig Dis Sci*. 2017;62:2348–56.

27. Mao L, Kitani A, Strober W, et al. The role of NLRP3 and IL-1beta in the pathogenesis of inflammatory bowel disease. *Front Immunol*. 2018;9:2566.
28. Bauer C, Duweil P, Lehr HA, et al. Protective and aggravating effects of Nlrp3 inflammasome activation in IBD models: influence of genetic and environmental factors. *Dig Dis*. 2012;30(Suppl 1):82–90.
29. Spalinger MR, Kasper S, Gottier C, et al. NLRP3 tyrosine phosphorylation is controlled by protein tyrosine phosphatase PTPN22. *J Clin Invest*. 2016;126:4388.
30. Mak'Anyengo R, Duweil P, Reichl C, et al. Nlrp3-dependent IL-1beta inhibits CD103+ dendritic cell differentiation in the gut. *JCI Insight* 2018;3.
31. Bersudsky M, Luski L, Fishman D, et al. Non-redundant properties of IL-1alpha and IL-1beta during acute colon inflammation in mice. *Gut*. 2014;63:598–609.
32. Liu L, Dong Y, Ye M, et al. The pathogenic role of NLRP3 inflammasome activation in inflammatory bowel diseases of both mice and humans. *J Crohns Colitis*. 2017;11:737–50.
33. Zhang J, Fu S, Sun S, et al. Inflammasome activation has an important role in the development of spontaneous colitis. *Mucosal Immunol*. 2014;7:1139–50.
34. Mao L, Kitani A, Similuk M, et al. Loss-of-function CARD8 mutation causes NLRP3 inflammasome activation and Crohn's disease. *J Clin Invest*. 2018;128:1793–806.
35. Yao X, Zhang C, Xing Y, et al. Remodelling of the gut microbiota by hyperactive NLRP3 induces regulatory T cells to maintain homeostasis. *Nat Commun*. 2017;8:1896.
36. Yilmaz B, Juillerat P, Oyas O, et al. Microbial network disturbances in relapsing refractory Crohn's disease. *Nat Med*. 2019;25:323–36.
37. O'Donnell S, Borowski K, Espin-Garcia O, et al. The Unsolved link of genetic markers and Crohn's disease progression: a North American cohort experience. *Inflamm Bowel Dis*. 2019;25:1541–9.
38. Lee JC, Biasci D, Roberts R, et al. Genome-wide association study identifies distinct genetic contributions to prognosis and susceptibility in Crohn's disease. *Nat Genet*. 2017;49:262–8.
39. Pierik M, Joossens S, Van Steen K, et al. Toll-like receptor-1, -2, and -6 polymorphisms influence disease extension in inflammatory bowel diseases. *Inflamm Bowel Dis*. 2006;12:1–8.
40. Lang BM, Biedermann L, van Haaften WT, et al. Genetic polymorphisms associated with smoking behaviour predict the risk of surgery in patients with Crohn's disease. *Aliment Pharmacol Ther*. 2018;47:55–66.
41. Coll RC, Robertson AA, Chae JJ, et al. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat Med*. 2015;21:248–55.
42. Jiang H, He H, Chen Y, et al. Identification of a selective and direct NLRP3 inhibitor to treat inflammatory disorders. *J Exp Med*. 2017;214:3219–38.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

