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

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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-1β 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
polymorphisms (SNPs) rs10733113, rs4353135 and rs55646866; however, 64–85% of the healthy European or Canadian populations 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 (CASP1). CASP1 is responsible for the conversion of pro-interleukin (IL)-1β 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-1β by lamina propria mononuclear cells isolated from inflamed IBD patients has been reported in several studies [4–6], and IL-1β 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-1β 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-TOF 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 \((\text{Score} = x \cdot \log(\text{OR rs4353135 T-allele}) + y \cdot \log(\text{OR rs55646866 C-allele}) + z \cdot \log(\text{OR rs10733113 G-allele}))\), 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()) [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 rs10733113 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 rs10733113, 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 |
|------------|-------|
|            | GG    | GT    |
| rs55646866: CC | 465 (59.2%) | 228 (29.9%) | 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%) |
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 rs43353135, 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 (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).

**Homzygous 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 | rs10733113 | rs10733113 | p value (Fisher or Kruskal–Wallis) |
|--------------------------|------------|------------|------------|-----------------------------------|
|                          | GG         | AG         | AA         |                                   |
| **Gender**               |            |            |            |                                   |
| 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%)|                                   |
| **Age at diagnosis [years]** |          |            |            |                                   |
| 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   |                                   |
| **Disease duration [years]** |          |            |            |                                   |
| 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   |                                   |
| **Complications***       |            |            |            |                                   |
| 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%)|                                   |
| **Fistula, abscess or anal fissure** |          |            |            |                                   |
| 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%)|                                   |
| **Focus on the 'yes':**  |            |            |            |                                   |
| 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                             |
| **Stenosis***           |            |            |            |                                   |
| 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%)|                                   |
| **Focus on the 'yes':**  |            |            |            |                                   |
| 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                             |
| **CDAI**—maximal value throughout follow-up | | | | |
| Median, q25–q75, | 63, 28–112, | 73, 20–76, | 76, 33–129, | 0.024 |
| Min–max                 | 2–435      | 6–81       | 0–479      |                                   |
| **Reported flare***     |            |            |            |                                   |
| 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%)|                                   |
| **Flare possibly or highly related to (focus on yes):** | | | | |
| 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                             |
| **Flare management**    |            |            |            |                                   |
| 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                             |
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. 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.

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

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) |
|--------------------------|--------------|--------------|--------------|------------------------------------|
| **Gender**               |              |              |              |                                    |
| 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%)   |                                    |
| **Age at diagnosis [years]** |              |              |              |                                    |
| 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                              |
| Disease duration [years] |              |              |              |                                    |
| 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                              |
| **Complications***       |              |              |              |                                    |
| No (n = 345)             | 247 (53.0%)  | 221 (53.9%)  | 50 (47.6%)   | 0.765                              |
| Yes (n = 636)            | 219 (47.0%)  | 189 (46.1%)  | 55 (52.4%)   |                                    |
| **Fistula, abscess or anal fissure*** |              |              |              |                                    |
| No (n = 518)             | 256 (54.9%)  | 239 (58.3%)  | 56 (53.3%)   | 0.513                              |
| Yes (n = 463)            | 210 (45.1%)  | 171 (41.7%)  | 49 (46.7%)   |                                    |
| **Focus on the 'yes'***  |              |              |              |                                    |
| 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                              |
| Surgery for fistula      | 114 (52.1%)  | 102 (54.0%)  | 30 (54.5%)   | 0.913                              |
| **Stenosis***            |              |              |              |                                    |
| 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%)   |                                    |
| **Focus on the 'yes'***  |              |              |              |                                    |
| 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                              |
| **CDAI—maximal value throughout follow-up** |              |              |              |                                    |
| Median, q25–q75, Min–max | 60, 26–109, 0–345 | 70, 31–126, 4–405 | 70, 34–118, 6–479 | 0.071                              |
| **Reported flare***      |              |              |              |                                    |
| 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%)   |                                    |
| **Flare possibly or highly related to (focus on yes):** |              |              |              |                                    |
| 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                              |
| **Flare management (focus on the yes):** |              |              |              |                                    |
| 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                              |
| **Focus on the hospitalizations:** |              |              |              |                                    |
| Total days of hosp.      | 7, 0–12,     | 5.5, 2–17,   | 12, 3–20,    | 0.646                              |
| Median, q25–q75, Min–max | 0–47         | 0–86         | 0–50         |                                    |
| Genotype        | rs55646866 CC | rs55646866 CT | rs55646866 TT | p value (Fisher or Kruskal–Wallis) |
|-----------------|---------------|---------------|---------------|-----------------------------------|
| **Gender**      |               |               |               |                                   |
| 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%)    |                                   |
| **Age at diagnosis [years]** |               |               |               |                                   |
| 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                             |
| **Disease duration [years]** |               |               |               |                                   |
| 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**                          |
| **Complications** |               |               |               |                                   |
| No (n = 345)    | 281 (35.8%)   | 56 (32.4%)    | 8 (34.8%)     | 0.727                             |
| Yes (n = 636)   | 504 (64.2%)   | 177 (67.6%)   | 15 (65.2%)    |                                   |
| **Fistula, abscess or anal fissure** |               |               |               |                                   |
| 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%)    |                                   |
| **Focus on the “yes”** |               |               |               |                                   |
| 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                             |
| **Stenosis**    |               |               |               |                                   |
| 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%)    |                                   |
| **Focus on the “yes”** |               |               |               |                                   |
| 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                             |
| **CDAI—maximal value throughout follow-up** |               |               |               |                                   |
| Median, q25–q75, Min–max | 64, 28–114, 0–435 | 76, 33–131, 6–479 | 64, 50–117, 6–280 | 0.108                             |
| **Reported flare** |               |               |               |                                   |
| 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%)    |                                   |
| **Flare possibly or highly related to (focus on yes)**: |               |               |               |                                   |
| 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                             |
| **Flare management (focus on the yes)**: |               |               |               |                                   |
| 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                             |
| **Focus on the hospitalizations**: |               |               |               |                                   |
| Total days of hosp. | 7.5, 2–16, 0–55 | 3.5, 0–17.5, 0–86 | 4, 3–5, 3–5 | 0.553                             |
| Median, q25–q75, Min–max |               |               |               |                                   |

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-1β 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-1β 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-1β secretion. In line with the proposed pro-inflammatory role for NLRP3 and IL-1β, such lower NLRP3 activity and IL-1β 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-1β secretion, thus that patients homozygous for all three major alleles would have the lowest IL-1β levels. Furthermore, polymorphisms in the IL1B gene have
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 inflammasome 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-1β levels are affected by cumulative effects of polymorphisms in IL1B itself and further genes involved in IL-1β 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-1β 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-1β in IBD is still elusive, as reviewed in detail recently [27]. Although consistently elevated levels of IL-1β secretion have been reported for both CD and UC patients, IL-1β 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 6** Demographic and clinical characteristics for the different genotypes of rs10733113 in UC patients

|                  | rs10733113 | rs10733113 | rs10733113 | p value (Fisher or Kruskal–Wallis) |
|------------------|------------|------------|------------|----------------------------------|
| **Gender**       |            |            |            |                                  |
| 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%) |                                  |
| **Age at diagnosis [years]** |            |            |            |                                  |
| 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                             |
| **Disease duration [years]** |            |            |            |                                  |
| 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                             |
| **Complications*** |            |            |            |                                  |
| 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%)|                                  |
| **MTWAI–maximal value throughout follow-up** |            |            |            |                                  |
| Median, q25–q75, Min–max | 4, 2–8.5, 0–19 | 7.5, 3–9, 0–13 | 5, 2–9, 0–17 | 0.704                            |
| **Reported flare*** |            |            |            |                                  |
| No (n = 284)     | 303 (58.7%)| 7 (50.0%)  | 96 (60.0%) | 0.753                            |
| Yes (n = 406)    | 213 (41.3%)| 7 (50.0%)  | 64 (40.0%) |                                  |
| **Flare possibly or highly related to (focus on yes):** |            |            |            |                                  |
| NSAIDs           | 19 (6.3%)  | 6 (4.2%)   | 4 (2.9%)   | 0.746                            |
| Antibiotics      | 6 (2.0%)   | 1 (1.4%)   | 1 (1.0%)   | 0.150                            |
| GI tract infection | 51 (16.8%) | 22 (29.9%) | 10 (10.4%) | 0.924                            |
| Other infection  | 29 (9.6%)  | 10 (14.3%) | 12 (8.5%)  |                                  |
| Treatment decr. / disc | 74 (24.4%) | 22 (29.9%) | 12 (8.5%)  |                                  |
| Other medication | 3 (1.0%)   | 5 (7.1%)   | 2 (2.9%)   | 0.864                            |
| **Flare management (focus on the yes):** |            |            |            |                                  |
| Hospitalization  | 39 (12.9%) | 7 (7.3%)   | 3 (3.1%)   | 0.278                            |
| Ambulatory       | 89 (29.4%) | 14 (14.6%) | 14 (14.6%) | 0.008                            |
| Surgery          | 16 (5.3%)  | 3 (3.1%)   | 9 (6.6%)   | 0.702                            |
| Drug therapy     | 271 (89.4%)| 90 (93.9%) | 32 (23.8%) | 0.367                            |
| **Focus on the hospitalizations:** |            |            |            |                                  |
| Total days of hosp. | 4, 0–16, 0–90 | 0, 0–3, 0–12 | 0.063 |                                  |

Bold indicates a significant p-value (p < 0.05)
Itani et al. describe a more severe course of disease of \textit{Nlrp3}−/− mice during oxazolone colitis \cite{11}, while Mak’Anyengo et al. observed reduced colitis in \textit{Nlrp3}−/− with the T-cell transfer colitis model \cite{30}. These findings may be explained by the different roles of IL-β during intestinal inflammation: Mak’Anyengo et al. showed NLRP3 dependent IL-1β 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-1β but IL-1α is the decisive pro-inflammatory mediator in the DSS colitis model, whereas IL-1β supports epithelial cell proliferation and restoration of colon barrier \cite{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 \textit{Il10}−/− mice that develop spontaneous colitis \cite{32,33}. In this model the pro-inflammatory role of IL-1β 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-1β inhibition \cite{34}.

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

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

|                         | rs4353135 | rs4353135 | rs4353135 | \(p\) value (Fisher or Kruskal–Wallis) |
|-------------------------|-----------|-----------|-----------|--------------------------------------|
|                         | TT        | GT        | GG        |                                       |
| **Gender**              |           |           |           |                                       |
| 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%) |                                       |
| **Age at diagnosis [years]** |       |           |           |                                       |
| 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                                |
| **Disease duration [years]** |       |           |           |                                       |
| 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                                |
| **Complications**       |           |           |           |                                       |
| 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%) |                                       |
| **MTWAI**               |           |           |           |                                       |
| Median, q25–q75, Min–max | 4, 2–9, 0–19 | 5, 2–9, 0–18 | 4, 2–7, 0–14 | 0.129                                |
| **Reported flare**      |           |           |           |                                       |
| 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%) |                                       |
| **Flare possibly or highly related to (focus on yes):** |           |           |           |                                       |
| 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 decr./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                                |
| **Flare management (focus on the yes):** |           |           |           |                                       |
| Hospitalization         | 24 (12.2%) | 20 (11.8%) | 2 (4.9%) | 0.443                                |
| Ambulatory              | 67 (34.2%) | 30 (17.8%) | 7 (17.1%) | \textbf{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                                |
| **Focus on the hospitalizations:** |           |           |           |                                       |
| Total days of hosp.     | 6.5, 2.5–18.5, 0–90 | 0, 0–7, 0–41 | 3, 0–6, 0–6 | \textbf{0.038}                       |
| Median, q25–q75, Min–max |           |           |           |                                       |

Bold indicates a significant \(p\)-value \((p < 0.05)\)
Ptpn22 [29]. As NLRP3 is activated by PTPN22-dephosphorylation, the Ptpn619W variant leads to increased NLRP3 activation and IL-1β 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-1β 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 SIBDUCS, 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

| Table 8 Demographic and clinical characteristics for the different genotypes of rs55646866 in UC patients |
|---------------------------------------------------------------|
| **rs55646866** |
| **CC** | **CT** | **TT** | **p value (Fisher or Kruskal–Wallis)** |
| **Gender** |
| 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%) | |
| **Age at diagnosis [years]** |
| 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** |
| Disease duration [years] |
| 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 |
| **Complications* ** |
| No (n = 286) | 244 (43.0%) | 39 (35.1%) | 3 (25.0%) | 0.160 |
| Yes (n = 404) | 322 (57.0%) | 72 (64.9%) | 9 (75.0%) | |
| **MTWI—maximal value throughout follow-up** |
| Median, q25-q75, Min-max | 5, 2–9, 0–19 | 5, 2–9, 0–17 | 1.5, 0–2.5, 0–14 | **0.009** |
| Reported flare* |
| 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%) | |
| Flare possibly or highly related to (focus on yes): |
| 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 decr./disc | 86 (25.5%) | 10 (16.1%) | 2 (28.6%) | 0.232 |
| Other medication | 10 (3.0%) | 4 (6.5%) | 0 (0%) | 0.413 |
| Flare management (focus on the yes): |
| 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 |
| Focus on the hospitalizations: |
| Total days of hosp. | 4, 0–14, – | 0, 0–6, | – | 0.225 |
| Median, q25-q75, Min-max | 0–90 | 0–12 | | |

Bold indicates a significant p-value (p < 0.05)
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-1β secretion, our findings suggest that during established CD the pro-inflammatory role of IL-1β 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-1β 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; SIBDUCS: 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; LR: Leucine rich repeat; NLR: NOD-like receptor; CDAl: Crohn’s disease activity index; MTWAI: Truelove and Witts activity index; NLRC4: Nucleotide-binding domain and leucine-rich repeat caspase recruitment domain 4.

Supplementary Information

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Additional file 1. Figure S1.
Additional file 2. Table S1.
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