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Corresponding author mail id: stb@mb.au.dk Polymorphisms in the NFkB, TNF-alpha, IL-1beta, and IL-18 pathways are associated with response to anti-TNF therapy in Danish inflammatory bowel disease patients

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Background: Anti-tumor necrosis factor-α (TNF-α) is used for the treatment of severe cases of inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC). However, one-third of the patients do not respond to the treatment. We have previously investigated whether single nucleotide polymorphisms (SNPs) in genes involved in inflammation were associated with response to anti-TNF therapy among patients with CD or UC.
Aim:A new cohort of patients was established for replication of the previous findings and to identify new SNPs associated with anti-TNF response.

Methods: Fifty-three SNPs assessed previously in cohort 1 (482CD and 256UC patients) were genotyped in cohort 2 (587CD and 458UC patients). The results were analyzed using logistic regression (adjusted for age and gender).

Results: Ten SNPs were associated with anti-TNF response either among patients with CD (*TNFRSF1A*(rs4149570)(OR:1.92,95%CI:1.02-3.60,p=0.04), *IL18*(rs187238)(OR:1.35,95%CI:1.00-1.82,p=0.05), and *JAK2*(rs12343867)(OR:1.35,95%CI:1.02-1.78,p=0.03)), UC (*TLR2*(rs11938228)(OR:0.55,95%CI:0.33-0.92,p=0.02), *TLR4*(rs5030728)(OR:2.23,95%CI:1.24-4.01,p=0.01) and (rs1554973)(OR:0.49,95%CI:0.27-0.90,p=0.02), *NFKBIA*(rs696)(OR:1.45,95%CI:1.06-2.00,p=0.02), and *NLRP3*(rs4612666)(OR:0.63,95%CI:0.44-0.91,p=0.01)) or in the combined cohort of patient with CD and UC (IBD) (*TLR4*(rs5030728)(OR:1.46,95%CI:1.01-2.11,p=0.04) and (rs1554973)(OR:0.80,95%CI:0.65-0.98,p=0.03), *NFKBIA*(rs696)(OR:1.25,95%CI:1.01-1.54,p=0.04), *NLRP3*(rs4612666)(OR:0.73,95%CI:0.57-0.95,p=0.02), *IL1RN*(rs4251961)(OR:0.81,95%CI:0.66-1.00,p=0.05), *IL18*(rs1946518)(OR:1.24,95%CI:1.01-1.53,p=0.04), and *JAK2*(rs12343867)(OR:1.24,95%CI:1.01-1.53,p=0.04)).

Conclusion: The results support that polymorphisms in genes involved in the regulation of the NFκB pathway (*TLR2*, *TLR4*, and *NFKBIA*), the TNF-α signaling pathway (*TNFRSF1A*), and other cytokine pathways (*NLRP3*, *IL1RN*, *IL18*, and *JAK2*) were associated with response to anti-TNF therapy. Our multi-SNP model predicted response rate of more than 82% (in 9% of the CD patients) and 75% (in 15% of the UC patients), compared to 71% and 64% in all CD and UC patients,respectively. More studies are warranted to predict response for use in the clinic.

Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are characterized by a dysregulated inflammatory response [1]. The diseases can be of great importance to the affected persons - in terms of reduced quality of life - and for the society - in terms of cost of lost labour due to sickness, treatment and medicine. Anti-tumor necrosis factor-α (anti-TNF) are therapeutic antibodies that block the binding of the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) to its receptors and limit downstream cell signaling pathways [2]. Anti-TNF can

also lead to apoptosis by binding to transmembrane TNF-α [3-5]. Anti-TNF is used for the treatment of severe cases of IBD, but approximately one-third of the patients benefit minimally or not at all from the treatment [6,7].

Pathogen-Associated Molecular Patterns (PAMPs) such as bacterial or viral DNA, flagellin or lipopolysaccharide (LPS) can bind to the membrane-bound Toll-like receptors (TLRs) leading to increased inflammation mediated by increased synthesis of a number of pro-inflammatory cytokines. PAMPs bound by the TLRs initiate a kinase cascade. This kinase cascade ultimately activates the IKK-complex, which phosphorylates and degrades the NFκB inhibitor IκBα [8]. The released NFκB is shuttled from the cytosol to the nucleus where it acts as a transcription factor which initiates expression of pro- and anti-inflammatory cytokines [9]. One of the pro-inflammatory cytokines activated by NFκB is TNF-α, which feedback stimulates NFκB by binding to TNF receptors (TNFR1 or TNFR2) resulting in a kinase cascade similar to, but distinct from, the canonical pathway induced by TLRs [8].

PAMPs may also be recognized by Nod-like receptors (NLRs), which are intracellular receptors. NLRP3 is a subunit of the inflammasome protein complexes and can activate pro- IL-1β and IL-18 which in turn can activate the synthesis of other cytokines [10,11].

By using a candidate gene approach of biological functional single nucleotide polymorphisms (SNPs) in selected pathways we have previously identified SNPs in genes involved in inflammation associated with response to anti-TNF therapy among patients with CD or UC [12,13]. We have now established a new cohort of patients with CD or UC treated with anti-TNF. The aim of the present study was to replicate our previous results in the new cohort, and to identify new SNPs associated with anti-TNF response using the increased statistical power of the combined cohort. The replication of the results in independent cohorts is the first step on the road to identify the biological pathways underlying treatment response and to develop algorithms predicting treatment effect for use in the clinic.

Materials and Methods

Cohort

This study was a replication of a previous study consisting of 482 CD and 256 UC Danish patients treated with anti-TNF (cohort 1) [12,13]. For this study, a replication cohort (cohort 2) of anti-TNF treated Danish patients with CD or UC was established. The cohort 2 was established using the same approach as was used for establishing cohort 1 [12,13]. Screening for *Mycobacterium tuberculosis* (TB) infection is routinely performed in Denmark before initiation of treatment with anti-TNF treatment. For the cohort 2, left over blood clots after whole blood analysis for TB were collected from 01.09.2009 to 31.01.2014 from patients screened for tuberculosis at Statens Serum Institut (Copenhagen, Denmark); the Department of Respiratory Diseases B and the Department of Clinical Microbiology, Aarhus University Hospital (Aarhus, Denmark); the Department of Clinical Biochemistry, Herlev and Gentofte Hospital (Hellerup, Denmark); the Department of Biochemistry, Hospital of Lillebaelt (Vejle, Denmark); and the Department of Biochemistry, Hospital of Slagelse (Slagelse, Denmark). Patients with intestinal diseases (ICD-10 code K50-K63) were identified by linking the unique personal identification number of Danish citizens (CPR-number) from each blood sample with the National Patient Registry. Patient records from 23 medical departments were examined and 587 CD and 458 UC patients treated with anti-TNF were identified. Figure 1 shows how cohort 2 was established. Patients on infliximab were routinely treated intravenously at the hospital whereas adalimumab was self-administered by the patients [14,15].

In this retrospective study treatment efficacy was assessed using the simple clinical 3-step scale [16-20] reflected the best response within 26 weeks after initiation. Patients with CD or UC with luminal disease were categorised as having: (A) no response, meaning no improvement or worsening of symptoms; (B) partial response, meaning some improvement of symptoms or reduction of steroid dose without worsening of symptoms; (C) response, meaning absence or almost absence of all clinical symptoms without increasing the steroid dose. Patients with fistulising CD were categorised as having: (A) no response, meaning no improvement or worsening of symptoms; (B) partial response, meaning reduced secretion or discomfort from fistulas or closure of one or some of the fistulas; (C) response, meaning closure of all fistulas evaluated by thumb pressure or no secretion. The medical doctors reviewing the patient records regarding treatment response to anti-TNF were blinded to genotyping results. Clinical and demographic characteristics of the patients with CD, UC, and IBD for the combined cohort (cohort 1 and 2 combined) are shown in Table 1, and data for cohort 1 and cohort 2 are shown in Supporting Table 1 and 2, respectively.

Genotyping

Biologically functional SNPs in genes involved in the NFκB, the TNF-α, and other cytokine pathways previously assessed in cohort 1 were genotyped in cohort 2 [12,13]. Most of the SNPs assessed have previously been examined by luciferase assay, enzyme-linked immunosorbent assay (ELISA), reverse transcriptase PCR, electrophoretic mobility shift assay (EMSA) or in flow cytometry assay to have a biological function which either increased or reduced gene activity [12,13]. A list of all SNPs studied, and the genotype distribution among patients with CD, UC, and IBD are presented in Supporting Table 3-5, respectively. Minor allele frequencies are presented in Supporting Table 6. DNA extraction (Maxwell 16 LEV Blood DNA Kit; Promega, Madison, WI, USA) was performed as described by *Bank et al.* [21]. Competitive Allele-Specific Polymerase chain reaction (KASP™), an end-point PCR technology, was used by LGC Genomics for genotyping (LGC Genomics, Hoddesdon, United Kingdom) (http://www.lgcgenomics.com/). As a quality control, all SNPs were replicated in 192 randomly selected samples (two 96-well plates minus two negative controls), yielding >99% identical genotypes. The average call rate for all SNPs was > 97%.

Power calculations

The Genetic Power Calculator was utilized for power analysis of discrete traits [22]. The 'high-risk allele frequency' was set to 0.10, 'prevalence' was set to 0.33, D-prime was set to 1, type I error rate was set to 0.05, and number of cases and control:case (responders:non&partial-responders) ratio was based on the actual distributions in the study group (found in Table 1).
The cohort 1, cohort 2, and the combined cohorts had more than 80% chance of detecting a dominant effect with an odds ratio (OR) of 1.5, 1.4, and 1.3, respectively.

Statistical analysis

Logistic regression adjusted for age at treatment and gender was used to compare genotypes between (a) responders versus non-responders; (b) responders versus non- and partial-responders to anti-TNF therapy among patients with CD, UC, and IBD (Supporting Table 7 and 8, respectively). Odds ratio unadjusted (crude) and adjusted for age at treatment, gender and smoking status between (a) responders versus non-responders; (b) responders versus non- and partial-responders to anti-TNF therapy among patients with CD, UC, and IBD were included in Supporting Table 9-12. A summary of SNPs associated with response to anti-TNF therapy and the biological effect of the studied SNPs are shown in Table 2.

A chi-square test or unpaired t-test was used to test for statistically significant difference in response between patients with CD and UC and for difference in secondary parameters (gender, age, location, smoking, concomitant medication, CRP, and F-cal) between responders and non-responders (Table 1) and for haplotype analysis (Supporting Table 13-15).
Statistical analyses were performed using STATA 15 (StataCorp LP, College Station, TX, USA).

Results

Study population

The clinical and demographic characteristics of the anti-TNF treated patients with CD (482 and 587 from cohort 1 and 2, respectively) and UC (256 and 458 from cohort 1 and 2, respectively) as well as CD and UC combined (IBD) are shown in Table 1. The percentage of of patients treated with infliximab, and adalimumab were 87%, and 13%, respectively. In the combined cohort, more females (16%) than males (9%) were non-responders among patients with CD (OR: 1.92, 95% CI: 1.31-2.82, p = 0.001). Young age at treatment was associated with beneficial response (p = 0.0007) among patients with CD. Never smoking was associated with beneficial response (OR: 2.27, 95% CI: 1.29-3.99, p = 0.005) among patients with CD.
Furthermore, the rate of non-response was higher among patients with UC than CD (23% (167 patients) and 13% (140 patients), respectively (OR: 1.98, 95% CI: 1.54-2.55, p = 0.0001)).

Polymorphisms associated with response in CD

In the combined cohort (cohort 1 and cohort 2 combined), the homozygous variant genotype of *TNFRSF1A* -609 G>T (rs4149570) (OR: 1.92, 95% CI: 1.02-3.60, p = 0.04) and the combined homozygous and the heterozygous variant genotypes of *IL18* -137 G>C (rs187238) (OR: 1.35, 95% CI: 1.00-1.82, p = 0.047) and *JAK2* T>C (rs12343867) (OR: 1.35, 95% CI: 1.02-1.78, p = 0.03) were associated with beneficial response among patients with CD (Supporting Table 7 and 8).

Polymorphisms associated with response in UC

In the combined cohort, the homozygous variant genotype of *TLR2* C>A (rs11938228) (OR: 0.55, 95% CI: 0.33-0.92, p = 0.02) and *TLR4* T>C (rs1554973) (OR: 0.49, 95% CI: 0.27-0.90, p = 0.02) and the combined homozygous and the heterozygous variant genotypes of *NLRP3* C>T (rs4612666) (OR: 0.63, 95% CI: 0.44-0.91, p = 0.01) were associated with non-response among patients with UC. The homozygous variant genotype of *TLR4* G>A (rs5030728) (OR: 2.23, 95% CI: 1.24-4.01, p = 0.01) and the combined homozygous and the heterozygous variant genotypes of *NFKBIA* 2758 G>A (rs696) (OR: 1.45, 95% CI: 1.06-2.00, p = 0.02) were associated with beneficial response among patients with UC (Supporting Table 7 and 8).

Polymorphisms associated with response in CD and UC combined (IBD)

In the combined cohort, the homozygous variant genotype of *TLR4* G>A (rs5030728) (OR: 1.46, 95% CI: 1.01-2.11, p = 0.04) and the combined homozygous and the heterozygous variant genotypes of *NFKBIA* 2758 G>A (rs696) (OR: 1.25, 95% CI: 1.01-1.54, p = 0.04), *JAK2* T>C (rs12343867) (OR: 1.24, 95% CI: 1.01-1.53, p = 0.04) and *IL18* -607 C>A (rs1946518) (OR: 1.24, 95% CI: 1.01-1.53, p = 0.04) were associated with beneficial response among patients with IBD. The combined homozygous and the heterozygous variant genotypes of *TLR4* T>C (rs1554973) (OR: 0.80, 95% CI: 0.65-0.98, p = 0.03), *NLRP3* C>T (rs4612666) (OR: 0.73, 95% CI: 0.57-0.95, p = 0.02) and *IL1RN* T>C (rs4251961) (OR: 0.81, 95% CI: 0.66-1.00, p = 0.049) were associated with non-response among patients with IBD (Supporting Table 7 and 8).

SNPs associated with anti-TNF response in CD, UC, or IBD patients and the biological interpretation of the associations are summarized in Table 2. A forest plot of the SNPs associated with anti-TNF response in CD, UC, or IBD patients is shown in Figure 2.

Haplotype analysis

Haplotype analysis of *TLR2, TLR4,* and *IL1B* are shown in Supporting Table 13-15, respectively. No associations were found in the haplotype analysis.

Predictive value

Sensitivity, specificity, positive (PPV) and negative predictive value (NPV) for risk sum (number of alleles) of the SNPs associated with drug response in CD or UC in the combined cohort is shown in Table 3 and 4, respectively. For CD, patients with a risk sum of 5 or 4-5 had a chance of 94% (16/17) and 82% (75/92), respectively, for being responders to anti-TNF treatment compared to

71% (760/1069) for all CD patients. This value applied to 2% (17/1069) and 9% (92/1069) of the CD patients, respectively. Similarly, for UC, patients with a risk sum of 0 or 0-2 had a chance of 89% (8/9) and 75% (79/105) for being responders to anti-TNF treatment compared to 64% (459/714) for all UC patients. This value applied to 1% (9/714) and 15% (105/714) of the UC patients, respectively.

Discussion

In this combined study (cohort 1 and 2 combined) 10 of the 53 SNPs assessed in the NFκB pathway (*TLR2* (rs11938228), *TLR4* (rs5030728 and rs1554973), and (*NFKBIA* (rs696)), the TNF-α pathway (*TNFRSF1A* (rs4149570)), and other pro-inflammatory pathways (*IL1RN* (rs4251961), *IL18* (rs1946518 and rs187238), *NLRP3* (rs4612666), and *JAK2* (rs12343867)), were associated with response to anti-TNF among patients with CD or UC. In this combined study 3 of the 15 SNPs associated with response after adjusting for age and gender in our previous studies of cohort 1[12,13] were replicated (*TLR4* (rs5030728 and rs1554973) and *TNFRSF1A* (rs4149570)). Associations with response to anti-TNF have previously been found for the *TLR2* (rs11938228), *IL1RN* (rs4251961), *IL18* (rs1946518), and *JAK2* (rs12343867) SNPs in unadjusted analysis or when adjusting for age, gender and smoking [12,13] with the odds ratio in the same direction as in this study of cohort 1 and 2 combined and adjusted for age and gender. Finally, novel associations were found in the NFκB pathway (*NFKBIA* (rs696)) and in other pro-inflammatory pathways (*NLRP3* (rs4612666) and *IL18* (rs187238)).

Based on our results we made a predictive model of response to anti-TNF, where we summarised the number of alleles (risk sum) of the SNPs associated with response in CD or UC (Table 3-4, and Supporting Figure 1-2). Although our predicitive model has limited clinical value as it is, it shows a clear trend of treatment response associated with allele count (risk sum) in the polygenetic predictive model in both cohort 1 and cohort 2, and in both CD and in UC patients (Supporting Table 16-19 and Supporting Figure 3-6). This indicates that with increasing number of SNPs associated with response used in the predictive model and with increasing number of patients included, that a clinical useful predictive model for anti-TNF treatment may be achieveable.

Hypothesis-free genome wide association studies (GWAS) and the establishment of more cohorts of anti-TNF treated patients is warranted to adequately predict response to anti-TNF for clinical use. Genetic studies of other diseases like breast cancer also started out with including a low number of patients and identifying few susceptibility SNPs. Now with incrieasing numbers of patients included in the studies and a change of focus from single SNP to creating algorithms including multiple susceptibility SNPs the models are increasingly getting value for clinical application [23].
Most of the SNPs assessed in our study have known biological effects thus allowing a biological interpretation of the observed associations based on increased or reduced gene activity as summarized in Table 2 and illustrated in Figure 3 [12,13,16,17,24-32]. Among patients with CD, the association observed for the *TNFRSF1A* (rs4149570) polymorphism indicates that increased expression of the TNF-α receptor 1 [33], and thus increased activity of the TNF-α pathway, was associated with beneficial response. Furthermore, the association observed for the *IL18* (rs187238) polymorphism indicates that increased IL-18 expression [34,35] was associated with non-response. In addition, the association observed for the *JAK2* (rs12343867) polymorphism indicates that increased JAK2 expression [36] was associated with non-response. The JAK2 kinase interacts with many different membrane receptors including the IFNG, IL12, and IL23 receptor [37,38]. The associations for the *IL18* (rs187238) and the *JAK2* (rs12343867) polymorphisms both indicate that genetically determined high activity of other cytokine pathways may be able to maintain the inflammation even after anti-TNF therapy and result in non-response.
Among patients with UC, the association observed for the *NFKBIA* (IκBα) (rs696) polymorphism indicates that reduced *NFKBIA* expression [39] was associated with non-response. IκBα acts as an inhibitor of the transcription factor NFκB which can activate many pro-inflammatory cytokines including IL-1β [9]. Thus, reduced activity of IκBα increases the activity of other cytokine pathways. Again, as among patients with CD, it could be speculated that high activity of other pro-inflammatory cytokines may be able to maintain the inflammation even after anti-TNF therapy and result in non-response.
The polymorphisms in *TLR2* (rs11938228) and *TLR4* (rs5030728 and rs1554973) associated with response among patients with UC have unknown biological effect but they underscore the importance of the NFκB pathway in response to anti-TNF therapy [40].
Furthermore, the association observed for the *NLRP3* (rs4612666) polymorphism indicates that increased NLRP3 expression [41] was associated with beneficial response. NLRP3 is involved in the inflammasome complex which can activate pro-IL-18. This may seem contradictory to the other

results in this study which indicates that increased IL-18 activity was associated with non-response. However, NLRP3 can also stimulate apoptosis as well as pyroptosis through activation of caspases [42,43]. Anti-TNF can induce apoptosis [3-5] and it could be speculated, that anti-TNF has a better effect among patients, whose pro-inflammatory secreting cells are genetically determined more prone for programmed cell death. In addition, the *NLRP3* (rs4612666) polymorphism has also been found to be associated with anti-TNF therapy among patients with rheumatoid arthritis [26].

In the combined cohort of patient with CD and UC (IBD), the same polymorphisms associated with response in CD (*JAK2* (rs12343867)) and UC (*NFKBIA* (rs696), *TLR4* (rs5030728 and rs1554973) and *NLRP3* (rs4612666)) were found. Furthermore, the association observed for the *IL18* (rs1946518) polymorphism indicates that increased IL-18 expression [34,35,44] was associated with non-response. As for the other polymorphism in *IL18* (rs187238), associated with response to anti-TNF among patient with CD, it could be speculated that genetically determined high activity of the *IL18* pathway may be able to maintain the inflammation even after anti-TNF therapy and result in non-response. In addition, the association observed for the *IL1RN* (rs4251961) (IL-1 receptor antagonist (IL-1RA) polymorphism indicates that reduced IL-1RA level [45] was associated with non-response. IL-1RA can bind to the IL-1β receptor and thereby inhibit IL-1β signaling [45]. It could be speculated that genetically determined high activity of the IL-1β pathway may be able to maintain the inflammation even after anti-TNF therapy and result in non-response. This interpretation is supported by another study which has reported an association between the C-allele of rs1143634 in *IL1B* and higher serum IL-1β level and a lower response rate to anti-TNF therapy among patients with CD [46]. It could be speculated that some patients who do not respond to anti-TNF therapy may have effect of IL-1β inhibitors or perhaps a combination treatment with anti-TNF and IL-1β inhibitors.

The results of this study should be interpreted with caution and should be evaluated in other cohorts of anti-TNF treated patients. In the light of the obtained P-values and the number of statistical tests performed, we cannot exclude that some of our positive findings may be due to chance (type I error). However, the direction of the odds ratio in all the 10 SNPs associated with response was biological plausible when interpretated based on the alleles effect on increased or reduced gene activity which is very unlikely if they were all chance findings ($p = 0.001$). We cannot exclude that associations were not identified due to insufficient statistical power. One of the SNPs associated with response in the cohort 1 study but not in this study was the *IL6* (rs10499563) polymorphism where the combined homozygous and the heterozygous variant genotypes were borderline associated with

beneficial response among patients with IBD (OR: 1.31, 95% CI: 0.99-1.71, p = 0.05). The variant allele has been shown to reduce IL-6 expression [47] which would indicate that patients with genetically determined high IL-6-driven inflammatory response were more likely to be non-responders to anti-TNF therapy. This supports that the activity of other cytokines than TNF-α are important predictors for the response to anti-TNF therapy.

A major strength of this study was that this clinically homogeneous and well-characterized cohort was rather large including 1783 patients with IBD treated with anti-TNF which gives the cohort study more than 80% power to detect an odds ratio of 1.3 assuming a minor allele frequency of 0.10. Furthermore, the found associations were biologically plausible. In addition, it is well-known that patients recruited to clinical trials are selected and may not represent "real" patients. This study collected clinical data from patients at 23 large gastroenterological centers at basic and specialized hospitals in Denmark. Thus, the patients are representative of Danish patients with severe IBD. Althought the collection of patients from the clinical setting may give an advantage in that the results may be readily transferable to the clinical setting, a drawback may be the definition of response which may be challenging. In this study, we have used the simple clinical 3-step scale to assess response, which has been used in other studies as well and it has turned out to be useful [16-20]. Another strength is that a high compliance was ensured in this study as 87% of the patients were treated with infliximab which is administered intraveneously at the hospital.

In conclusion, the results indicate that polymorphisms in genes involved in the regulation of the NFκB pathway (*TLR2*, *TLR4*, and *NFKBIA*), the TNF-α signaling pathway (*TNFRSF1A*), and other cytokine pathways (*NLRP3*, *IL1RN*, *IL18*, and *JAK2*) were associated with response to anti-TNF therapy. Overall, the associations in *TNFRSF1A*, *IL1RN* and *IL18* indicate that patients with genetically determined high TNF-driven inflammatory response are most likely to benefit from anti-TNF therapy while patients with genetically determined high IL-1β or IL-18-driven inflammatory response seem to be less likely to benefit from anti-TNF therapy, perhaps because these cytokines may be able to maintain the inflammation even after anti-TNF therapy and thus result in non-response. More studies including larger cohorts and using genome-wide analysis are warranted to develop genetic biomarkers to predict response to anti-TNF for use in the clinic.

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Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Regional Ethics Committees of Central (M20100153) and Southern (S-20120113) Denmark and the Danish Data Protection Agency of Central (RM: J. 2010-41-4719) and Southern (RSD: 2008-58-035) Denmark. The Ethics Committees gave an exemption for obtaining written informed consent.

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Authorship Statement

Guarantor: SB is the submission's guarantor.

Author contributions: SB, ACB, PSA, ABB, SBS, JS, UBV and VA designed the research study and PSA, OKA, JB, JBB, NKP, AG, RA, DNR, CHG, SR, JG, DSHF, KL, ShR, BOL, BKR, SA, MON, EKNB, AP, MJ, DRR, LA, MJA, AF, LM, AH, CSR, ES, NEH, RGN, FS, KC, JS, LL, MRA, IB, RBD, HJH, BAN, MT and AG collected the materials. SB analysed the data and wrote the first draft. All authors approved the final version of the manuscript.

Conflict of Interest:

Vibeke Andersen received compensation as a consultant for MSD (Merck) and Janssen, and as a member of the advisory board for MSD (Merck). Mette Julsgaard has served on the advisory board of Tillotts Pharma and Janssen, has received consultation fees from Ferring, and has received speaker's fee from MSD, Ferring, UCB and Takeda. Katrine Carlsen had received a research grants from MSD and Tillotts pharma, Denmark. The other authors declare no conflicts of interest.

Availability of data and material

Data is stored at Odense Patient data Explorative Network (OPEN). Bona fide researchers can apply to use the dataset by applying to open.rsyd.dk.

Table 1: Clinical and demographic characteristics for the combined Danish cohort (cohort 1 and cohort 2 combined) for anti-tumor necrosis factor alpha (TNF-α) naïve patients with Crohn's disease, ulcerative colitis and inflammatory bowel disease treated with anti-TNF.

	Crohn's disease (CD)				Ulcerative colitis (UC)				Inflammatory bowel disease (IBD)			
Characteristics	Responders	Non-responders	Non&Partial responders	p[a]	Responders	Non-responders	Non&Partial responders	p[a]	Responders	Non-responders	Non&Partial responders	p[a]
Efficacy - no (%)	761 (71)	140 (13)	308 (29)	-	459 (64)	167 (23)	255 (36)	-	1220 (68)	307 (17)	563 (32)	-
Gender - no (%)												
Male	356 (75)	44 (9)	117 (25)		221 (64)	77 (22)	122 (36)		577 (71)	121 (15)	239 (29)	
Female	405 (68)	96 (16)	191 (32)	8*10^-4	238 (64)	90 (24)	133 (36)	0.72	643 (66)	186 (19)	324 (34)	0.06
Age, years												
Age at treatment, median (range)	33 (3-79)	40 (16-77)	38 (15-77)	7*10^-4	38 (4-84)	37 (15-81)	38 (7-81)	0.68	35 (3-84)	38 (15-81)	38 (7-81)	7*10^-3
Anti-TNF antibody treatment												
Infliximab	624 (82)	103 (74)	240 (78)		442 (96)	151 (90)	234 (92)		1066 (87)	254 (83)	474 (84)	
Adalimumab	135 (18)	36 (26)	67 (22)	0.03	11 (2)	16 (10)	21 (8)	3*10^-4	146 (12)	52 (17)	88 (16)	0.01
Golimumab	2 (0)	1 (0)	1 (0)	-	6 (1)	0 (0)	0 (0)	-	8 (1)	1 (0)	1 (0)	-
Location - no (%)												
Ileal (L1)	198 (26)	47 (34)	84 (27)	0.08	-	-	-	-	198 (26)	47 (34)	84 (27)	0.08
Colonic (L2)	249 (33)	39 (28)	98 (32)	0.28	-	-	-	-	249 (33)	39 (28)	98 (32)	0.28
Ileocolonic (L3)	263 (35)	43 (31)	97 (31)	0.38	-	-	-	-	263 (35)	43 (31)	97 (31)	0.38
Data not available	51 (7)	11 (8)	29 (9)	-	-	-	-	-	51 (7)	11 (8)	29 (9)	-
Location - no (%)												
Proctitis (E1)	-	-	-	-	83 (18)	33 (20)	48 (19)	0.64	83 (18)	33 (20)	48 (19)	0.64
Left side (E2)	-	-	-	-	144 (31)	56 (34)	83 (33)	0.63	144 (31)	56 (34)	83 (33)	0.63
Extensive (E3)	-	-	-	-	185 (40)	61 (37)	89 (35)	0.41	185 (40)	61 (37)	89 (35)	0.41
Data not available	-	-	-	-	47 (10)	17 (10)	35 (14)	-	47 (10)	17 (10)	35 (14)	-

Smoking history, at treatment – no (%)												
Current smoker	192 (25)	37 (26)	85 (28)	0.83	32 (7)	6 (4)	11 (4)	0.13	224 (18)	43 (14)	96 (17)	0.24
Former smoker	75 (10)	11 (8)	30 (10)	0.53	96 (21)	35 (21)	55 (22)	1.00	171 (14)	46 (15)	85 (15)	0.82
Never smoker	163 (21)	15 (11)	44 (14)	0.01	91 (20)	35 (21)	52 (20)	0.82	254 (21)	50 (16)	96 (17)	0.22
Data not available	331 (43)	77 (55)	149 (48)	-	240 (52)	91 (54)	137 (54)	-	571 (47)	168 (55)	286 (51)	
Concomitant medication – no (%)												
Azathioprine	297 (39)	51 (36)	138 (45)	0.57	138 (30)	47 (28)	73 (29)	0.69	435 (36)	98 (32)	211 (37)	0.32
5-aminosalicylates	54 (7)	12 (9)	21 (7)	0.60	178 (39)	72 (43)	105 (41)	0.36	232 (19)	84 (27)	126 (22)	0.01
Glucocorticoids	228 (30)	57 (41)	118 (38)	0.01	228 (50)	87 (52)	128 (50)	0.65	456 (37)	144 (47)	246 (44)	0.02
Methotrexate	21 (3)	4 (3)	11 (4)	1.00	5 (1)	1 (1)	2 (1)	0.69	26 (2)	5 (2)	13 (2)	0.60
Antibiotics	35 (5)	12 (9)	28 (9)	0.06	20 (4)	11 (7)	16 (6)	0.30	55 (5)	23 (7)	44 (8)	0.11
C-reactive protein (CRP) and F-calprotectin – no (%)												
≥ 25% decrease in CRP within 22 weeks. Baseline CRP ≥ 20 mg/L	167/169 (99)	15/24 (63)	47/58 (81)	7*10 ^-8	84/84 (100)	15/28 (54)	27/42 (64)	1*10 ^-9	251/253 (99)	30/52 (58)	74/100 (74)	3*10 ^-17
≥ 25% decrease in F-cal within 22 weeks. Baseline F-cal > 200 mg/kg	158/163 (97)	13/20 (65)	44/56 (79)	4*10 ^-5	106/108 (98)	13/24 (54)	29/45 (64)	5*10 ^-8	264/271 (97)	26/44 (59)	73/101 (72)	3*10 ^-12

[a]p-values were calculated by comparing responders versus non-responders.

Table 2: Biological interpretation of the single nucleotide polymorphisms (SNPs) associated with anti-TNF response in Crohn's disease (CD), ulcerative colitis (UC) or inflammatory bowel disease (IBD) patients in the combined Danish cohort (cohort 1 and cohort 2 combined).

Gene	Rs-number	Pathway	Model	OR (95% CI)	P-value	Effect of minor-allele	Biological interpretation
				Crohn's disease (CD)			
TNFRSF1A	rs4149570	Cytokines	TT vs GG	1.92 (1.02-3.60)	0.04[a]	-609T increase expression in PBMC [33]	Increased TNF-α receptor 1 expression was associated with beneficial response. This indicates that genetically determined high TNF-driven inflammatory response was associated with beneficial response.
IL18	rs187238	Cytokines	GC vs GG	1.35 (1.00-1.82)	0.047[b]	-137C reduce IL-18 level in serum [34] and expression in PBMC [35]	Reduced IL-18 expression was associated with beneficial response. This indicates that genetically determined high IL18-driven inflammatory response was associated with non-response.
JAK2	rs12343867	Cytokines	TC or CC vs TT	1.35 (1.02-1.78)	0.03[b]	rs12343867C reduce expression in granulocytes and K562 cells [36]	Reduced JAK2 expression was associated with beneficial response. The JAK2 kinase interacts with many different membrane receptors including the IFNG, IL12, and IL23 receptor [37,38]. This indicates that genetically determined high activity of other pro-inflammatory pathways was associated with non-response.
				Ulcerative colitis (UC)			
TLR2	rs11938228	Pathogen recognition	AA vs CC	0.55 (0.33-0.92)	0.02[b]	Unknown [40]	-
TLR4	rs5030728	Pathogen recognition	AA vs GG	2.23 (1.24-4.01)	0.01[b]	Unknown [40]	-
TLR4	rs1554973	Pathogen recognition	CC vs TT	0.49 (0.27-0.90)	0.02[b]	Unknown [40]	-
NFKBIA	rs696	Pathogen recognition	GA or AA vs GG	1.45 (1.06-2.00)	0.02[b]	2758A increase expression in HCT116, HT29 and SW480 cells [39]	Increased *NFKBIA* expression was associated with beneficial response. IκBα acts as an inhibitor of the transcription factor NFκB which can activate many pro-inflammatory cytokines including IL-1β [9]. This indicates that genetically determined low NFκB activity, and thus low activity of other pro-inflammatory pathways, was associated with beneficial response.
NLRP3	rs4612666	Apoptosis	CT or TT	0.63 (0.44-0.91)	0.01[a]	rs4612666T reduce expression	Reduced *NLRP3* expression was associated with non-

			vs CC			in THP-1 cells [41]	response. NLRP3 is involved in the inflammasome complex and can also stimulate apoptosis through activation of caspases [42,43]. Anti-TNF can induce apoptosis [3-5] and it could be speculated, that anti-TNF has a better effect among patients, whose pro-inflammatory secreting cells are genetically determined more prone for programmed cell death.
Inflammatory bowel disease (IBD)							
TLR4	rs5030728	Pathogen recognition	AA vs GG	1.46 (1.01-2.11)	0.04[b]	Unknown [40]	Same as for UC.
TLR4	rs1554973	Pathogen recognition	TC or CC vs TT	0.80 (0.65-0.98)	0.03[b]	Unknown [40]	Same as for UC.
NFKBIA	rs696	Pathogen recognition	GA or AA vs GG	1.25 (1.01-1.54)	0.04[b]	2758A increase expression in HCT116, HT29 and SW480 cells [39]	Same as for UC.
IL1RN	rs4251961	Cytokines	TC or CC vs TT	0.81 (0.66-1.00)	0.049[b]	rs4251961C reduce IL-1RA level in Serum and HEK293T cells [45,48]	Reduced IL-1 receptor antagonist (IL-1RA) level was associated with non-response. IL-1RA can bind to the IL-1β receptor and thereby inhibit IL-1β signaling [45]. This indicates that genetically determined high IL-1β-driven inflammatory response was associated with non-response.
IL6	rs10499563	Cytokines	CT or CC vs TT	1.31 0.99-1.71)	0.052[a]	–6331C reduce expression in serum [47]	Reduced IL-6 expression was borderline associated with beneficial response. This indicates that genetically determined high IL6-driven inflammatory response was associated with non-response.
IL18	rs1946518	Cytokines	GT or TT vs GG	1.24 (1.01-1.53)	0.04[b]	-607AA reduce IL-18 level in serum [34,44] and expression in PBMC [35]	Reduced IL-18 expression was associated with beneficial response. This indicates that genetically determined high IL18-driven inflammatory response was associated with non-response.
NLRP3	rs4612666	Apoptosis	CT or TT vs CC	0.73 (0.57-0.95)	0.02[a]	rs4612666T reduce expression in THP-1 cells [41]	Same as for UC.
JAK2	rs12343867	Cytokines	TC or CC vs TT	1.24 (1.01-1.53)	0.04[b]	rs12343867C reduce expression in granulocytes and K562 cells [36]	Same as for CD.

PBMC: peripheral blood mononuclear cell.

[a]Responder vs non-responders.

[b]Responder vs non- and partial.responders.

Table 3: Odds ratio (OR), 95% confidence interval (95% CI), sensitivity, specificity, positive (PPV) and negative predictive value (NPV) for the risk sum (number of alleles) of the three single nucleotide polymorphisms associated with drug response in Crohns disease in the combined cohort.

Risk sum (number of alleles)[a]	Responders	Non&partial-responders	OR	95% CI	p-value	Sensitivity	Specificity	PPV	NPV
5 (N = 17)	16	1	1	-		-	-	94	6
4 (N =75)	59	16	4.34	(0.53-35.24)	0.18	98	0	79	21
3 (N = 223)	166	57	5.49	(0.71-42.36)	0.08	90	6	74	26
2 (N = 305)	215	90	6.70	(0.88-51.27)	0.049	68	24	70	30
1 (N = 294)	202	92	7.29	(0.95-55.78)	0.028	40	53	69	31
0 (N = 154)	102	52	8.16	(1.05-63.22)	0.024	13	83	66	34

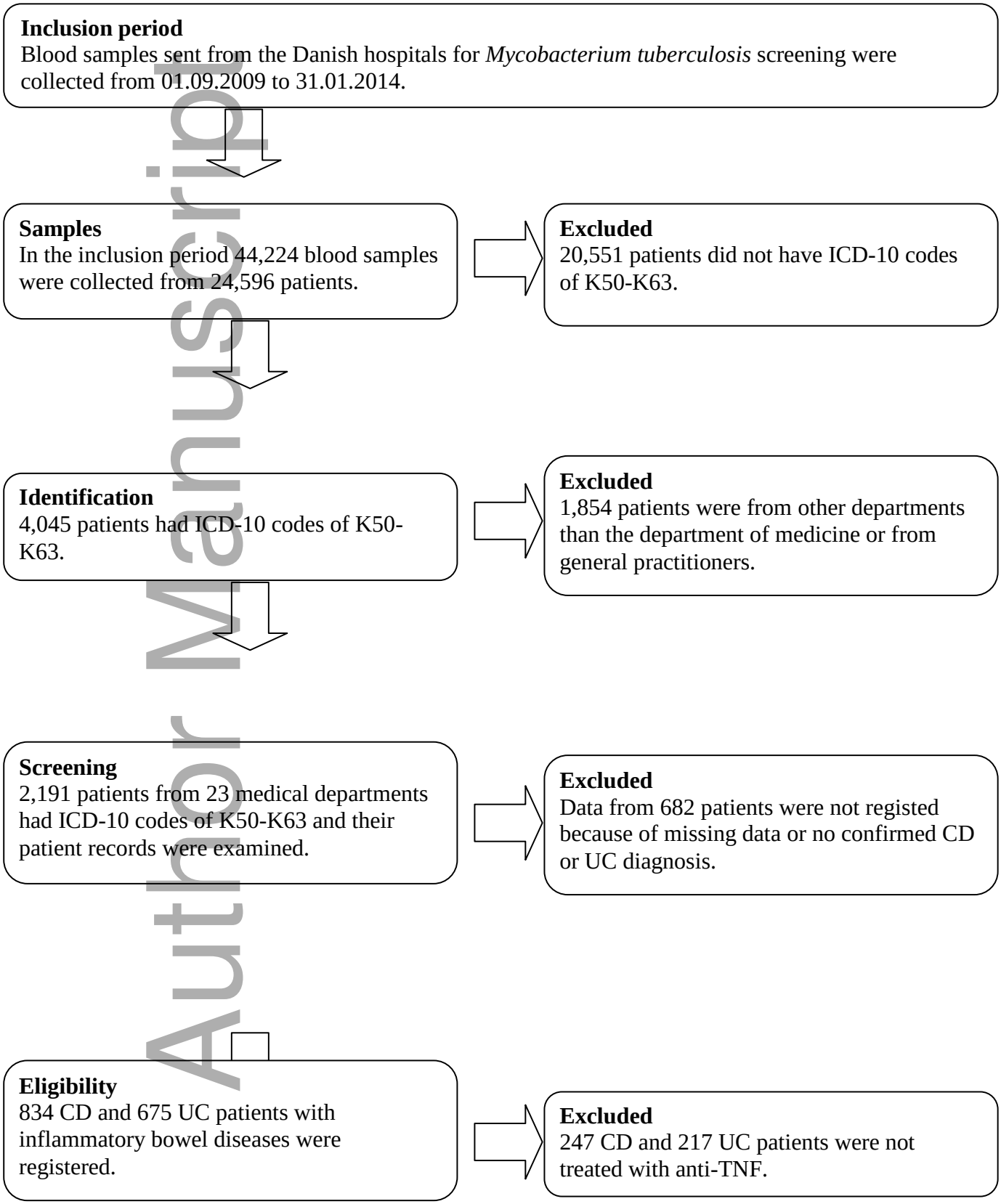
[a]The risk sum was calculated by summarising the number of risk alleles for the three SNPs associated with drug response in Crohns disease (TNFRSF1A, rs4149570 (GG=0; GT=1; TT=2), IL18, rs187238 (GG=0; GC=1; CC=2) and JAK2, rs12343867 (TT=0; TC=1; CC=2)).

Table 4: Odds ratio (OR), 95% confidence interval (95% CI), sensitivity, specificity, positive (PPV) and negative predictive value (NPV) for the risk sum (number of alleles) of the five single nucleotide polymorphisms associated with drug response in ulcerative colitis in the combined cohort.

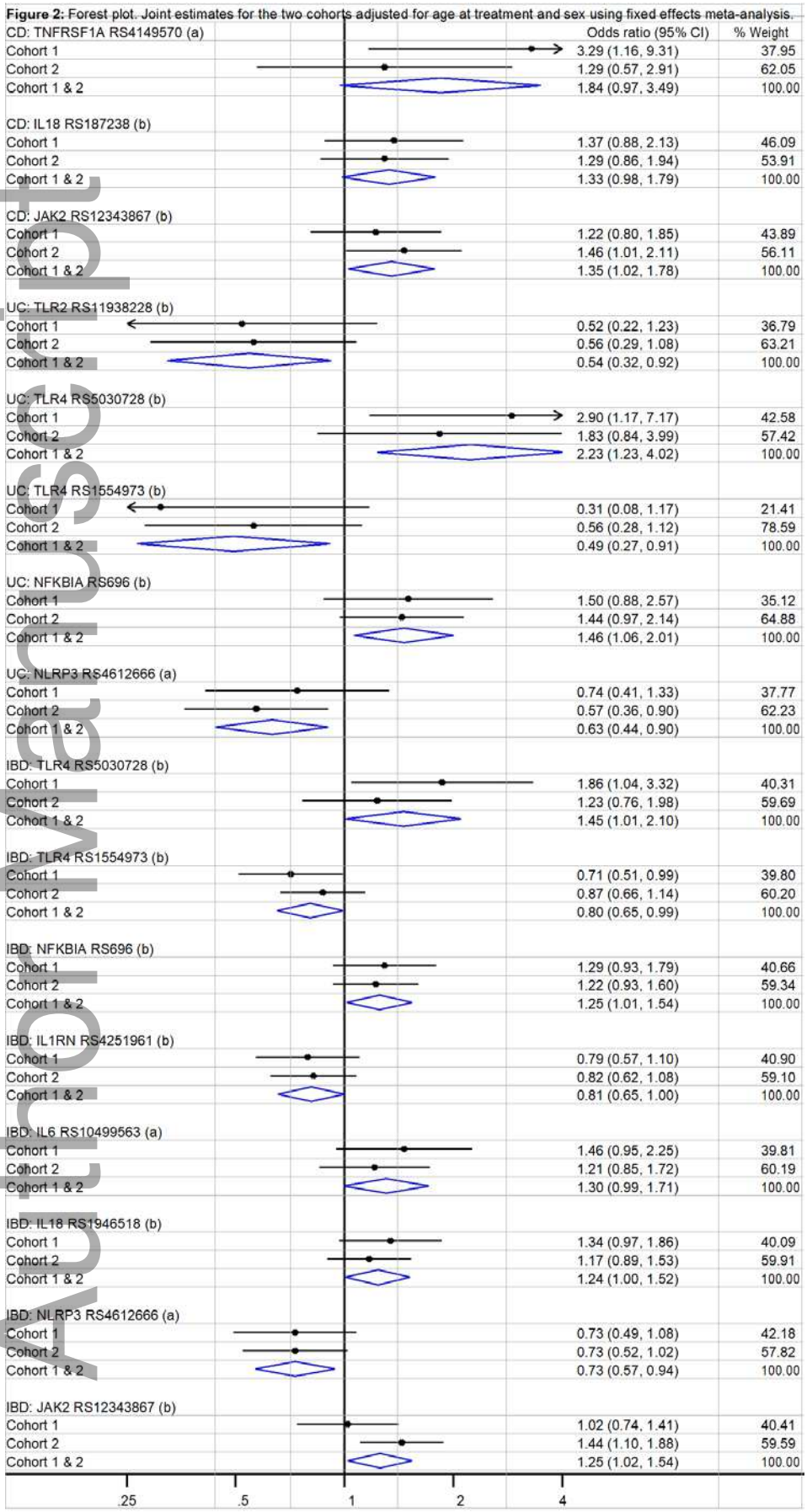
Risk sum (number of alleles)[a]	Responders	Non&partial-responders	OR	95% CI	p-value	Sensitivity	Specificity	PPV	NPV
8 (N = 12)	3	9	1	-	-	-	-	25	75
7 (N = 42)	18	24	2.25	(0.53-9.52)	0.33	99	4	43	57
6 (N = 88)	49	39	3.77	(0.96-14.87)	0.064	95	13	56	44
5 (N = 151)	90	61	4.43	(1.15-17.01)	0.031	85	28	60	40
4 (N =180)	122	58	6.31	(1.65-24.19)	0.0042	65	52	68	32
3 (N = 136)	98	38	7.74	(1.99-30.12)	0.0017	39	75	72	28
2 (N = 66)	46	20	6.90	(1.69-28.21)	0.0048	17	90	70	30
1 (N = 30)	25	5	15.00	(2.96-75.91)	0.00063	7	98	83	17
0 (N = 9)	8	1	24.00	(2.06-279.64)	0.0075	2	100	89	11

[a]The risk sum was calculated by summarising the number of risk alleles for the five SNPs associated with drug response in ulcerative colitis (TLR2, rs11938228 (CC=0; AC=1; AA=2), TLR4, rs5030728 (GG=2; GA=1; AA=0), TLR4, rs1554973 (TT=0; TC=1; CC=2), NFKBIA, rs696 (GG=2; GA=1; AA=0) and NLRP3, rs4612666 (CC=0; CT=1; TT=2).

Figure 1. Flow diagram showing how cohort 2 was established.



Included
587 CD and 458 UC patients were treated with anti-TNF. The patients had not previously been treated with anti-TNF.
Response was achieved by 69% (406) and 65% (296) of the patients with CD and UC respectively.
Non- or partial-response was achived by 31% (181) and 35% (162) of the patients with CD and UC, respectively, within 26 weeks of treatment.



a: Responders vs non-responders. b: Responders vs non- and partial responders.

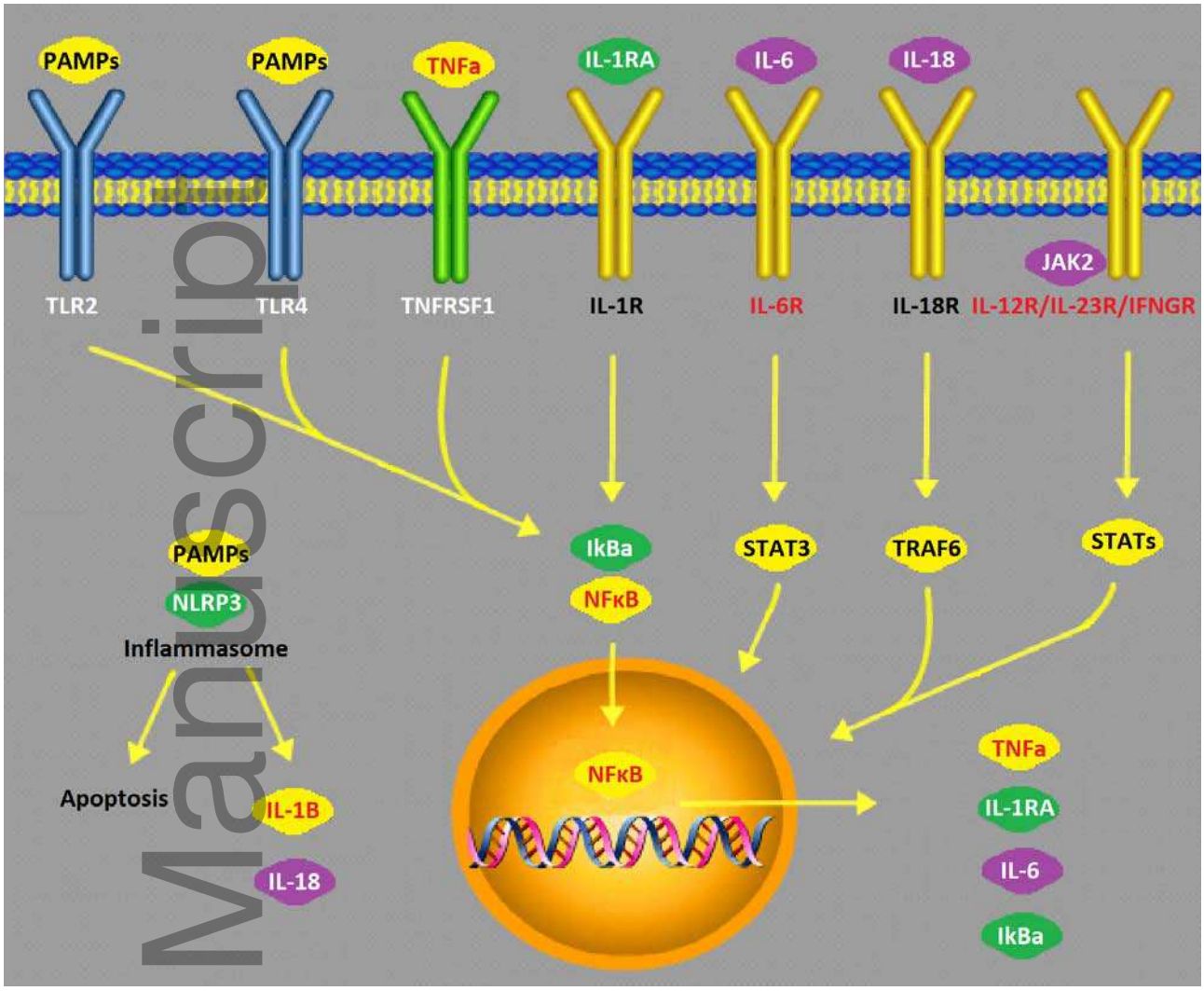


Figure 3: Simplified overview of the pathways assessed.

Polymorphisms in genes associated or not associated with response to anti-TNF therapy among patients with Crohn's disease or ulcerative colitis are written in white and red, respectively. Genes not studied are written in black.

Green: Increased gene/protein activity was associated with beneficial response

Purple: Increased gene/protein activity was associated with non-response.

Blue: The biological effect was unknown.

Yellow: The genes were not studied (black text) or were not associated with response (red text).

References

1. Podolsky DK. Inflammatory bowel disease. *The New England journal of medicine.* 2002;347(6):417-429.
2. Knight DM, Trinh H, Le J, et al. Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. *Molecular immunology.* 1993;30(16):1443-1453.
3. Siegel SA, Shealy DJ, Nakada MT, et al. The mouse/human chimeric monoclonal antibody cA2 neutralizes TNF in vitro and protects transgenic mice from cachexia and TNF lethality in vivo. *Cytokine.* 1995;7(1):15-25.
4. Scallon BJ, Moore MA, Trinh H, Knight DM, Ghrayeb J. Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. *Cytokine.* 1995;7(3):251-259.
5. ten Hove T, van Montfrans C, Peppelenbosch MP, van Deventer SJ. Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. *Gut.* 2002;50(2):206-211.
6. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *The New England journal of medicine.* 2005;353(23):2462-2476.
7. Mascheretti S, Hampe J, Kuhbacher T, et al. Pharmacogenetic investigation of the TNF/TNF-receptor system in patients with chronic active Crohn's disease treated with infliximab. *The pharmacogenomics journal.* 2002;2(2):127-136.
8. Verstrepen L, Bekaert T, Chau TL, Tavernier J, Chariot A, Beyaert R. TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. *Cellular and molecular life sciences : CMLS.* 2008;65(19):2964-2978.
9. http://www.bu.edu/nf-kb/gene-resources/target-genes/.
10. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. *Nature.* 2012;481(7381):278-286.
11. Chang HD, Radbruch A. The pro- and anti-inflammatory potential of interleukin-12. *Annals of the New York Academy of Sciences.* 2007;1109:40-46.

12. Bank S, Andersen PS, Burisch J, et al. Associations between functional polymorphisms in the NFkappaB signaling pathway and response to anti-TNF treatment in Danish patients with inflammatory bowel disease. *The pharmacogenomics journal.* 2014;14(6):526-534.
13. Bank S, Andersen PS, Burisch J, et al. Genetically determined high activity of IL-12 and IL-18 in ulcerative colitis and TLR5 in Crohns disease were associated with non-response to anti-TNF therapy. *The pharmacogenomics journal.* 2017.
14. Dignass A, Van Assche G, Lindsay JO, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *Journal of Crohn's & colitis.* 2010;4(1):28-62.
15. Dignass A, Lindsay JO, Sturm A, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *Journal of Crohn's & colitis.* 2012;6(10):991-1030.
16. Bank S, Andersen PS, Burisch J, et al. Effectiveness of anti-tumour necrosis factor-alpha therapy in Danish patients with inflammatory bowel diseases. *Danish medical journal.* 2015;62(3).
17. Bank S. A cohort of anti-TNF treated Danish patients with inflammatory bowel disease, used for identifying genetic markers associated with treatment response. *Danish medical journal.* 2015;62(5).
18. Ljung T, Karlen P, Schmidt D, et al. Infliximab in inflammatory bowel disease: clinical outcome in a population based cohort from Stockholm County. *Gut.* 2004;53(6):849-853.
19. Caspersen S, Elkjaer M, Riis L, et al. Infliximab for inflammatory bowel disease in Denmark 1999-2005: clinical outcome and follow-up evaluation of malignancy and mortality. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2008;6(11):1212-1217; quiz 1176.
20. Cohen RD, Tsang JF, Hanauer SB. Infliximab in Crohn's disease: first anniversary clinical experience. *The American journal of gastroenterology.* 2000;95(12):3469-3477.
21. Bank S, Nexo BA, Andersen V, Vogel U, Andersen PS. High-quality and -quantity DNA extraction from frozen archival blood clots for genotyping of single-nucleotide polymorphisms. *Genetic testing and molecular biomarkers.* 2013;17(6):501-503.
22. http://zzz.bwh.harvard.edu/gpc/cc2.html.

23. Mavaddat N, Pharoah PD, Michailidou K, et al. Prediction of breast cancer risk based on profiling with common genetic variants. *Journal of the National Cancer Institute.* 2015;107(5).
24. Bank S, Skytt Andersen P, Burisch J, et al. Polymorphisms in the inflammatory pathway genes TLR2, TLR4, TLR9, LY96, NFKBIA, NFKB1, TNFA, TNFRSF1A, IL6R, IL10, IL23R, PTPN22, and PPARG are associated with susceptibility of inflammatory bowel disease in a Danish cohort. *PloS one.* 2014;9(6):e98815.
25. Bank S, Andersen PS, Burisch J, et al. Polymorphisms in the Toll-Like Receptor and the IL-23/IL-17 Pathways Were Associated with Susceptibility to Inflammatory Bowel Disease in a Danish Cohort. *PloS one.* 2015;10(12):e0145302.
26. Sode J, Vogel U, Bank S, et al. Anti-TNF treatment response in rheumatoid arthritis patients is associated with genetic variation in the NLRP3-inflammasome. *PloS one.* 2014;9(6):e100361.
27. Sode J, Vogel U, Bank S, et al. Genetic Variations in Pattern Recognition Receptor Loci Are Associated with Anti-TNF Response in Patients with Rheumatoid Arthritis. *PloS one.* 2015;10(10):e0139781.
28. Sode J, Vogel U, Bank S, et al. Confirmation of an IRAK3 polymorphism as a genetic marker predicting response to anti-TNF treatment in rheumatoid arthritis. *The pharmacogenomics journal.* 2016.
29. Loft ND, Skov L, Iversen L, et al. Associations between functional polymorphisms and response to biological treatment in Danish patients with psoriasis. *The pharmacogenomics journal.* 2017.
30. Bek S, Nielsen JV, Bojesen AB, et al. Systematic review: genetic biomarkers associated with anti-TNF treatment response in inflammatory bowel diseases. *Alimentary pharmacology & therapeutics.* 2016;44(6):554-567.
31. Bek S, Bojesen AB, Nielsen JV, et al. Systematic review and meta-analysis: pharmacogenetics of anti-TNF treatment response in rheumatoid arthritis. *The pharmacogenomics journal.* 2017;17(5):403-411.
32. Loft ND, Skov L, Rasmussen MK, et al. Genetic polymorphisms associated with psoriasis and development of psoriatic arthritis in patients with psoriasis. *PloS one.* 2018;13(2):e0192010.

33. Wang GB, Li CR, Yang J, Wen PQ, Jia SL. A regulatory polymorphism in promoter region of TNFR1 gene is associated with Kawasaki disease in Chinese individuals. *Human immunology.* 2011;72(5):451-457.
34. Jaiswal PK, Singh V, Srivastava P, Mittal RD. Association of IL-12, IL-18 variants and serum IL-18 with bladder cancer susceptibility in North Indian population. *Gene.* 2013;519(1):128-134.
35. Dziedziejko V, Kurzawski M, Paczkowska E, Machalinski B, Pawlik A. The impact of IL18 gene polymorphisms on mRNA levels and interleukin-18 release by peripheral blood mononuclear cells. *Postepy higieny i medycyny doswiadczalnej (Online).* 2012;66:409-414.
36. Spasovski V, Tosic N, Nikcevic G, et al. The influence of novel transcriptional regulatory element in intron 14 on the expression of Janus kinase 2 gene in myeloproliferative neoplasms. *Journal of applied genetics.* 2013;54(1):21-26.
37. Hoeve MA, Savage ND, de Boer T, et al. Divergent effects of IL-12 and IL-23 on the production of IL-17 by human T cells. *European journal of immunology.* 2006;36(3):661-670.
38. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol.* 2004;75(2):163-189.
39. Song S, Chen D, Lu J, et al. NFkappaB1 and NFkappaBIA polymorphisms are associated with increased risk for sporadic colorectal cancer in a southern Chinese population. *PloS one.* 2011;6(6):e21726.
40. Gast A, Bermejo JL, Claus R, et al. Association of inherited variation in Toll-like receptor genes with malignant melanoma susceptibility and survival. *PloS one.* 2011;6(9):e24370.
41. Hitomi Y, Ebisawa M, Tomikawa M, et al. Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma. *The Journal of allergy and clinical immunology.* 2009;124(4):779-785.e776.
42. D'Osualdo A, Reed JC. NLRP1, a regulator of innate immunity associated with vitiligo. *Pigment cell & melanoma research.* 2012;25(1):5-8.
43. Sagulenko V, Thygesen SJ, Sester DP, et al. AIM2 and NLRP3 inflammasomes activate both apoptotic and pyroptotic death pathways via ASC. *Cell death and differentiation.* 2013;20(9):1149-1160.
44. Chen DY, Chen YM, Chen HH, Hsieh CW, Lin CC, Lan JL. Functional association of interleukin 18 gene -607 (C/A) promoter polymorphisms with disease course in Chinese

patients with adult-onset Still's disease. *The Journal of rheumatology.* 2009;36(10):2284-2289.

45. Rafiq S, Stevens K, Hurst AJ, et al. Common genetic variation in the gene encoding interleukin-1-receptor antagonist (IL-1RA) is associated with altered circulating IL-1RA levels. *Genes and immunity.* 2007;8(4):344-351.
46. Lacruz-Guzman D, Torres-Moreno D, Pedrero F, et al. Influence of polymorphisms and TNF and IL1beta serum concentration on the infliximab response in Crohn's disease and ulcerative colitis. *European journal of clinical pharmacology.* 2013;69(3):431-438.
47. Smith AJ, D'Aiuto F, Palmen J, et al. Association of serum interleukin-6 concentration with a functional IL6 -6331T>C polymorphism. *Clinical chemistry.* 2008;54(5):841-850.
48. Carrol ED, Payton A, Payne D, et al. The IL1RN promoter rs4251961 correlates with IL-1 receptor antagonist concentrations in human infection and is differentially regulated by GATA-1. *Journal of immunology (Baltimore, Md : 1950).* 2011;186(4):2329-2335.