The Pharmacogenomics Journal (2014) 14, 526–534

www.nature.com/tpj

OPEN

ORIGINAL ARTICLE

Associations between functional polymorphisms in the NFκB signaling pathway and response to anti-TNF treatment in Danish patients with inflammatory bowel disease

S Bank[1,2], PS Andersen[3], J Burisch[4], N Pedersen[4], S Roug[5], J Galsgaard[6], SY Turino[7], JB Brodersen[8], S Rashid[9], BK Rasmussen[10], S Avlund[11], TB Olesen[12], HJ Hoffmann[13], MK Thomsen[14], VØ Thomsen[15], M Frydenberg[16], BA Nexø[2], J Sode[17,18,19], U Vogel[20] and V Andersen[1,17,21,22]

Antitumor necrosis factor-α (TNF-α) is used for 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. Genetic markers may predict individual response to anti-TNF therapy. Using a candidate gene approach, 39 mainly functional single nucleotide polymorphisms (SNPs) in 26 genes regulating inflammation were assessed in 738 prior anti-TNF-naive Danish patients with IBD. The results were analyzed using logistic regression (crude and adjusted for age, gender and smoking status). Nineteen functional polymorphisms that alter the NFκB-mediated inflammatory response (*TLR2* (rs3804099, rs11938228, rs1816702, rs4696480), *TLR4* (rs5030728, rs1554973), *TLR9* (rs187084, rs352139), *LY96* (MD-2) (rs11465996), *CD14* (rs2569190), *MAP3K14* (NIK) (rs7222094)), TNF-α signaling (*TNFA* (TNF-α) (rs361525), *TNFRSF1A* (TNFR1) (rs4149570), *TNFAIP3*(A20) (rs6927172)) and other cytokines regulated by NFκB (*IL1B* (rs4848306), *IL1RN* (rs4251961), *IL6* (rs10499563), *IL17A* (rs2275913), *IFNG* (rs2430561)) were associated with response to anti-TNF therapy among patients with CD, UC or both CD and UC ($P \leqslant 0.05$). In conclusion, the results suggest that polymorphisms in genes involved in activating NFκB through the Toll-like receptor (TLR) pathways, genes regulating TNF-α signaling and cytokines regulated by NFκB are important predictors for the response to anti-TNF therapy among patients with IBD. Genetically strong TNF-mediated inflammatory response was associated with beneficial response. In addition, the cytokines IL-1β, IL-6 and IFN-γ may be potential targets for treating patients with IBD who do not respond to anti-TNF therapy. These findings should be examined in independent cohorts before these results are applied in a clinical setting.

The Pharmacogenomics Journal (2014) **14,** 526–534; doi:10.1038/tpj.2014.19; published online 29 April 2014

INTRODUCTION

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are characterized by a dysregulated inflammatory response.[1] The transcription factor NFκB is a central regulator of inflammation and regulates the expression of more than 150 genes including *TNFA*, *TNFAIP3*, *TLR2*, *TLR9*, *CD14*, *NFKBIA*, *NFKB1*, *IL1B*, *IL1RN*, *IL6*, *IL10*, *IL17A* and *IFNG*.[2] NFκB can be activated by Toll-like receptors (TLRs), which recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, such as bacterial or viral DNA, flagellin or lipopolysaccharide (LPS). For example, LPS from Gram-negative bacterial cell membranes can be bound by CD14 in the presence of LPS-binding protein, which together with MD-2 interacts with TLR4. TLR4 is a membrane-bound protein, which on LPS stimuli forms homodimers and activates an intracellular kinase cascade. This kinase cascade ultimately activates the IKK complex, which phosphorylates and degrades the NFκB inhibitor IκBα.[3] NFκB is shuttled from the cytosol to the nucleus where it initiates expression of pro- and anti-inflammatory cytokines (Figure 1).

One of the pro-inflammatory cytokines activated by NFκB is tumor necrosis factor-α (TNF-α), whose 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.[3]

The TNF-α level is increased in the blood, stool and intestinal tissue from patients with CD and UC, and therefore it has been a target for medical treatment.[4–6] Infliximab and adalimumab are therapeutic antibodies that block the binding of TNF-α to its cell-surface receptors and limit downstream cell signaling pathways.[7] These antibodies are 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.[8,9]

[1]Medical Department, Viborg Regional Hospital, Viborg, Denmark; [2]Biomedicine, University of Aarhus, Aarhus, Denmark; [3]Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark; [4]Department of Gastroenterology, Herlev Hospital, Herlev, Denmark; [5]Department of Gastroenterology, Hvidovre Hospital, Hvidovre, Denmark; [6]Medical Department, Køge Hospital, Køge, Denmark; [7]Medical Department, Hillerød Hospital, Hillerød, Denmark; [8]Medical Department, Sydvestjysk Hospital, Esbjerg, Denmark; [9]Medical Department, Bispebjerg Hospital, Bispebjerg, Denmark; [10]Medical Department, Nykøbing Falster Hospital, Nykøbing Falster, Denmark; [11]Medical Department V, Aarhus University Hospital, Aarhus, Denmark; [12]Medical Department, Slagelse Hospital, Slagelse, Denmark; [13]Department of Respiratory Diseases B, Institute for Clinical Medicine, Aarhus University Hospital, Aarhus, Denmark; [14]Department of Clinical Microbiology, Aarhus University Hospital, Aarhus, Denmark; [15]International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark; [16]Section of Biostatistics, Department of Public health, Aarhus University, Aarhus, Denmark; [17]Institute of Regional Health Research, University of Southern Denmark, Odense, Denmark; [18]Clinical Biochemistry, Immunology & Genetics, Statens Serum Institut, Copenhagen, Denmark; [19]Department of Rheumatology, Frederiksberg Hospital, Frederiksberg, Denmark; [20]National Research Centre for the Working Environment, Copenhagen, Denmark; [21]Organ Centre, Hospital of Southern Jutland Aabenraa, Aabenraa, Denmark and [22]OPEN Odense Patient data Explorative Network, Odense University Hospital, Odense, Denmark. Correspondence: Dr S Bank, Medical Department, Viborg Regional Hospital, Heibergs Allé 4, Viborg DK-8800, Denmark.
E-mail: stb@mb.au.dk
Received 21 October 2013; revised 1 March 2014; accepted 10 March 2014; published online 29 April 2014

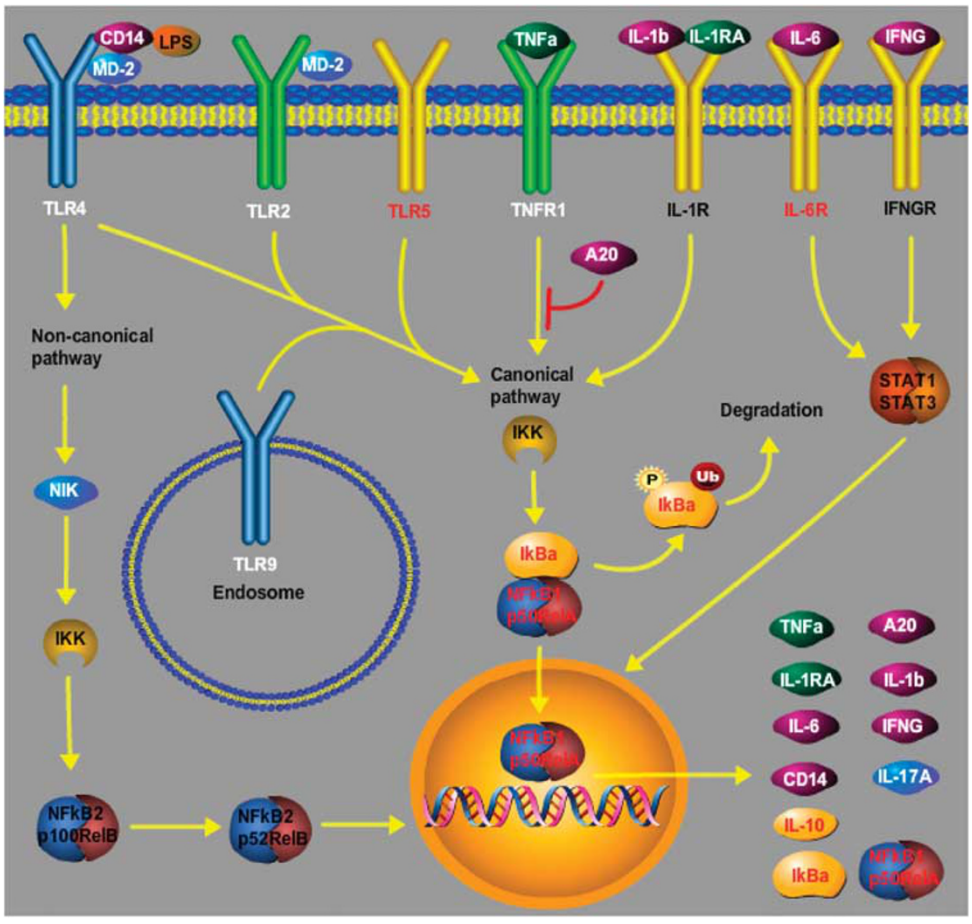

Figure 1. Simplified overview of the NFκB pathway highlighting the genes that were studied. Polymorphisms in genes associated or not associated with response to anti-TNF therapy among patients with IBD are written in white and red, respectively. Genes not studied are written in black. Increased gene/protein activity was associated with beneficial response (green) or nonresponse (purple). The biological effect was unclear (TLR4, TLR9, NIK) or showed opposite direction of effect among patients with CD and UC (MD-2) (blue). A significant association was only seen in CD14 and IL-1RA (inhibitor of IL-1β signaling) among patients with UC. CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.

We wanted to investigate whether there were genetic differences between responders and nonresponders to anti-TNF therapy among patients with IBD. Therefore, we assessed 39 mainly functional polymorphisms in genes involved in inflammation, in particular the NFκB pathway, in a cohort of 738 Danish IBD patients previously naive to anti-TNF therapy. Knowing the biological effect of the studied polymorphisms allows a biological interpretation of the associations in the candidate genes. The genes studied are involved in regulation of the NFκB pathway (*TLR2*, *TLR4*, *TLR5*, *TLR9*, *LY96*, *CD14*, *MAP3K14*, *SUMO4*, *NFKBIA* and *NFKB1*), TNF-α signaling (*TNFA*, *TNFRSF1A* and *TNFAIP3*), cytokines regulated by NFκB (*IL1B*, *IL1RN*, *IL6*, *IL10*, *IL17A* and *IFNG*) and other genes involved in regulation of inflammation (*IL4R*, *IL6R*, *IL23R*, *TGFB1*, *PTPN22*, *PPARG* and *NLRP3*).

MATERIALS AND METHODS

Cohort

A prior anti-TNF-naive Danish cohort of patients with IBD was established. Blood samples retrieved as part of the routine screening for latent *Mycobacterium tuberculosis* at Statens Serum Institut (SSI, Copenhagen, Denmark) and the Department of Respiratory Diseases B, and the Department Clinical Microbiology, Aarhus University Hospital (Aarhus, Denmark) were collected from 01 September 2009 to 30 March 2011 (9217 patients). 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 (2659 patients). Patient records from 18 medical departments were examined (1378 patients) and identified 738 previously anti-TNF-naive ethnic Danish patients with IBD. Treatment efficacy using the simple three-step scale[10–12] reflected the maximum response within 22 weeks after initiation.

Genotyping

DNA was extracted from cryopreserved blood clots by using the Maxwell 16 Blood purification kit (Promega, Madison WI, USA) according to the manufacturers' instructions with a median yield of 4.90 μg (range 0.8–25 μg) per 300 μl total blood.[13] Competitive Allele-Specific Polymerase chain reaction (KASP), an end-point PCR technology, was used by LGC Genomics for genotyping (LGC Genomics, Hoddesdon, UK) (http://www.lgcgenomics.com/).

The single nucleotide polymorphisms (SNPs) studied were *TLR2* (rs4696480, rs1816702, rs11938228, rs3804099), *TLR4* (rs12377632, rs5030728, rs1554973), *TLR5* (rs5744168), *TLR9* (rs187084, rs352139), *LY96* (MD-2) (rs11465996), *CD14* (rs2569190), *MAP3K14* (NIK) (rs7222094), *SUMO4* (rs237025), *NFKBIA* (IκBα) (rs696, rs17103265), *NFKB1* (NFκB1) (rs28362491), *TNFA* (TNF-α) (rs1800629, rs1800630, rs1799724, rs361525), *TNFRSF1A* (TNFR1) (rs4149570), *TNFAIP3* (A20) (rs6927172), *IL1B* (IL-1β) (rs1143623, rs4848306, rs1143627), *IL1RN* (IL-1RA) (rs4251961), *IL4R* (rs1805010),

IL6 (rs10499563), *IL6R* (rs4537545), *IL10* (rs1800872, rs3024505), *IL17A* (rs2275913), *IL23R* (rs11209026), *IFNG* (IFN-γ) (rs2430561), *TGFB1* (TGF-β1) (rs1800469), *PTPN22* (rs2476601), *PPARG* (PPAR-γ) (rs1801282) and *NLRP3* (rs4612666).

Genotyping of *TNFA* (TNF-α) –857 C>T (rs1799724) and –863 C>A (rs1800630) failed due to their close proximity to each other. All genotyping of –857 C>T (rs1799724) either failed or were erroneously genotyped as homozygous wild type when the patients were carriers of the AA genotype of –863 C>A (rs1800630) due to genotyping bias.

The 39 SNPs were replicated in 94 randomly selected samples and yielded >99% identical genotypes. The studied SNPs had a minor allele frequency of 0.05–0.48.

Linkage disequilibrium was calculated using the Genome-wide LInkage DisEquilibrium Repository and Search Engine (GLIDER) software (http://www.sanger.ac.uk/resources/software/gliders/). Haplotypes were inferred manually.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local regional ethics committees (M20100153 and S-20120113) and the Danish Data Protection Agency (J. 2010-41-4719 and 2008-58-035).

Power analysis

The Genetic Power Calculator was utilized for power analysis of discrete traits.[14] The 'high-risk allele frequency' was set to 0.05, 0.25 and 0.48, the '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 ratio was based on Table 1. This cohort study had more than 80% chance of detecting a dominant effect with an odds ratio (OR) of 1.9, 1.5 and 1.6 for CD, 2.1, 1.7 and 1.9 for UC and 1.6, 1.4 and 1.5 for IBD with high-risk allele frequencies of 0.05, 0.25 and 0.48, respectively.

Statistical analysis

Logistic regression, crude and adjusted for age, gender and smoking status, was used to compare genotypes among responders versus nonresponders and responders versus non- and partial responders to anti-TNF therapy (Supplementary Tables 1–3).

A χ^2-test or unpaired *t*-test was used to test for statistically significant difference in response between patients with CD and UC, for difference in secondary parameters between responders and nonresponders (Table 1), and for haplotype analysis (Supplementary Tables 4–7).

To achieve more statistical power, analyses for associations between polymorphisms and treatment response in relation to IBD and for haplotype analyzed were performed with the combined CD and UC data set.

Statistical analyses were performed using STATA version 11 (STATA, College Station, TX, USA).

RESULTS

Study population

The clinical and demographic characteristics of the 482 and 256 prior anti-TNF-naive patients with CD and UC, respectively, are shown in Table 1. More females (17%) than males (9%) were nonresponders among patients with CD (OR: 2.05, 95% CI: 1.16–3.64, $P=0.01$) and a decrease in CRP level by more than 25% was associated with beneficial response among patients with IBD when only considering CRP $\geqslant 20\,mg\,l^{-1}$ pretreatment (OR: 2.01, 95% CI: 1.09–3.72, $P=0.02$). Furthermore, 14% (66 patients) and 24% (62 patients) were nonresponders among patients with CD and UC, respectively (OR: 0.48, 95% CI: 0.33–0.72, $P=0.001$). Young age at diagnosis was borderline associated with beneficial response ($P=0.06$).

Polymorphisms associated with response in CD

In a dominant model, the homozygous variant genotype of *TNFRSF1A* –609G>T (rs4149570) (OR_{adj}: 2.39, 95% CI: 1.03–5.57, $P=0.04$), the heterozygous genotype of *TLR4* G>A (rs5030728) (OR_{adj}: 3.34, 95% CI: 1.32–8.47, $P=0.01$) and both the homozygous and the heterozygous variant genotypes of *TLR2* 597T>C (rs3804099) (OR_{adj}: 2.02, 95% CI: 1.17–3.49, $P=0.01$), *TLR2* C>T (rs1816702) (OR_{adj}: 2.02, 95% CI: 1.04–3.95, $P=0.04$), *LY96* (MD-2) –1625 C>G (rs11465996) (OR_{unadj}: 1.73, 95%CI: 1.01–2.95, $P=0.04$) and *IFNG* 874T>A (rs2430561) (OR_{adj}: 1.97, 95% CI: 1.13–3.42, $P=0.02$) were associated with beneficial response among patients with CD (Supplementary Table 1).

In a recessive model, the homozygous variant genotypes of *TLR4* G>A (rs5030728) (OR_{adj}: 0.38, 95% CI: 0.15–0.96, $P=0.04$) and *TLR9* 1174G>A (rs352139) (OR_{adj}: 0.38, 95% CI: 0.16–0.94, $P=0.04$) were associated with nonresponse and the homozygous variant genotype of *TNFRSF1A* –609G>T (rs4149570) (OR_{adj}: 5.16, 95% CI: 1.14–23.39, $P=0.03$) was associated with beneficial response among patients with CD (Supplementary Table 1).

The variant allele of the polymorphisms has been shown to decrease TNF-α, IL-1β and IL-6 levels (*TLR2* 597T>C (rs3804099)), increase TLR2 (*TLR2* C>T (rs1816702)), MD-2 and TNF-α levels (*LY96* –1625 C>G (rs11465996)), increase *TNFRSF1A* expression (*TNFRSF1A* –609G>T (rs4149570)) and decrease IFN-γ level (*IFNG* 874T>A (rs2430561)) (Table 2).

The biological effect of the polymorphism *TLR4* G>A (rs5030728) and *TLR9* 1174G>A (rs352139) was unknown.

Thus, genetically determined increased TLR2 level (rs1816702), MD-2 level (rs11465996), *TNFRSF1A* (TNFR1) expression (rs4149570) and genetically determined decreased IL-1β (rs3804099), IL-6 (rs3804099) and IFN-γ (rs2430561) levels were associated with beneficial response among patients with CD. The genetically determined effect of TNF-α was inconclusive among patients with CD, as one polymorphism indicated that genetically determined increased TNF-α level (rs11465996) and another polymorphism indicated that decreased TNF-α level (along with decreased IL-1β and IL-6 levels) (rs3804099) were associated with beneficial response.

Polymorphisms associated with response in UC

In a dominant model, the homozygous variant genotype of *TLR4* G>A (rs5030728) (OR_{unadj}: 2.89, 95% CI: 1.17–7.12, $P=0.02$) and both the homozygous and the heterozygous variant genotypes of *IL1B* –3737G>A (rs4848306) (OR_{adj}: 2.69, 95% CI: 1.04–6.94, $P=0.04$) and *IL6* –6331T>C (rs10499563) (OR_{adj}: 3.60, 95% CI: 1.39–9.29, $P=0.01$) were associated with beneficial response among patients with UC. The homozygous variant genotype of *TLR2* A>T (rs4696480) (OR_{unadj}: 0.47, 95% CI: 0.23–0.95, $P=0.04$) and both the homozygous and the heterozygous variant genotypes of *TLR2* C>A (rs11938228) (OR_{unadj}: 0.49, 95% CI: 0.26–0.90, $P=0.02$), *LY96* (MD-2) –1625 C>G (rs11465996) (OR_{adj}: 0.32, 95% CI: 0.14–0.75, $P=0.01$), *CD14* –159G>A (rs2569190) (OR_{unadj}: 0.54, 95% CI: 0.30–0.98, $P=0.04$), *TNFAIP3* (A20) C>G (rs6927172) (OR_{adj}: 0.34, 95% CI: 0.13–0.90, $P=0.03$), *IL1RN* T>C (rs4251961) (OR_{adj}: 0.42, 95% CI: 0.18–0.98, $P=0.04$) and *IL17A* 197G>A (rs2275913) (OR_{adj}: 0.42, 95% CI: 0.18–1.00, $P=0.05$) were associated with nonresponse (Supplementary Table 2).

In a recessive model, the homozygous variant genotype of *TLR2* 597T>C (rs3804099) (OR_{unadj}: 2.47, 95% CI: 0.98–6.23, $P=0.05$) and the homozygous variant genotype of *TLR4* G>A (rs5030728) (OR_{unadj}: 2.59, 95% CI: 1.08–6.18, $P=0.03$) were associated with beneficial response among patients with UC. The homozygous variant genotype of *TLR2* C>A (rs11938228) (OR_{adj}: 0.26, 95% CI: 0.08–0.92, $P=0.04$) and the homozygous variant genotype of *TLR2* A>T (rs4696480) (OR_{adj}: 0.29, 95% CI: 0.12–0.70, $P=0.01$) were associated with nonresponse (Supplementary Table 2).

The variant allele of the polymorphisms has been shown to decrease TNF-α, IL-1β and IL-6 levels (*TLR2* 597T>C (rs3804099)), increase MD-2 and TNF-α levels (*LY96* –1625 C>G (rs11465996)), CD14 level (*CD14* –159G>A (rs2569190)), increase *TNFAIP3* expression (*TNFAIP3* C>G (rs6927172)), decrease *IL1B* transcription (*IL1B* –3737G>A (rs4848306)), decrease IL-1RA level

Table 1. Clinical and demographic characteristics for antitumor necrosis factor alpha (TNF-α)-naive inflammatory bowel disease patients treated with anti-TNF

Characteristics	*CD*				*UC*				*IBD*			
	Responders	*Nonresponders*	*Non- and partial responders*	P-*value*	*Responders*	*Nonresponders*	*Non- and partial responders*	P-*value*	*Responders*	*Nonresponders*	*Non- and partial responders*	P-*value*
Efficacy—no. (%)	355 (74)	66 (14)	127 (26)	—	163 (64)	62 (24)	93 (36)	—	518 (70)	128 (17)	220 (30)	—
Gender—no. (%)												
Male	161 (78)	19 (9)	46 (22)		81 (65)	27 (22)	44 (35)		242 (73)	46 (14)	90 (27)	
Female	194 (71)	47 (17)	81 (29)	0.01	82 (63)	35 (27)	49 (37)	0.46	276 (68)	82 (20)	130 (32)	0.03
Age, years												
Age at diagnosis, median (range)	24 (7–77)	27 (14–70)	25 (10–70)	0.11	31 (12–81)	31 (11–77)	31 (4–77)	0.80	26 (7–81)	29 (11–77)	27 (4–77)	0.06
Location—no. (%)												
Ileal (L1)	90 (25)	24 (36)	36 (28)	0.07	—	—	—	—	—	—	—	—
Colonic (L2)	121 (34)	23 (35)	47 (37)	1.00	—	—	—	—	—	—	—	—
Ileocolonic (L3)	127 (36)	15 (23)	37 (29)	0.05	—	—	—	—	—	—	—	—
Distribution—no. (%)												
Proctitis (E1)	—	—	—	—	21 (13)	14 (23)	17 (18)	0.10	—	—	—	—
Left side (E2)	—	—	—	—	76 (47)	28 (45)	42 (45)	0.88	—	—	—	—
Extensive (E3)	—	—	—	—	53 (33)	15 (24)	22 (24)	0.26	—	—	—	—
Data not available	17 (5)	4 (6)	7 (6)	—	13 (8)	5 (8)	12 (13)	—	—	—	—	—
Smoking history—no. (%)												
Current smoker	99 (28)	22 (33)	43 (34)	0.46	18 (11)	1 (2)	2 (2)	0.03	117 (23)	23 (18)	45 (20)	0.14
Former smoker	39 (11)	5 (8)	12 (9)	0.51	36 (22)	13 (21)	20 (22)	1.00	75 (14)	18 (14)	32 (15)	1.00
Never smoker	92 (26)	11 (17)	32 (25)	0.12	39 (24)	13 (21)	19 (20)	0.60	131 (25)	24 (19)	51 (23)	0.11
Data not available	125 (35)	28 (42)	40 (31)	—	70 (43)	35 (56)	52 (56)	—	195 (38)	63 (49)	92 (42)	—
Concomitant medication—no. (%)												
Azathioprine	102 (29)	15 (23)	42 (33)	0.37	29 (18)	13 (21)	21 (23)	0.70	131 (25)	28 (22)	63 (29)	0.43
5-aminosalicylates	28 (8)	5 (8)	7 (6)	1.00	61 (37)	24 (39)	33 (35)	0.88	89 (17)	29 (23)	40 (18)	0.16
Glucocorticoids	105 (30)	24 (36)	45 (35)	0.31	83 (51)	31 (50)	43 (46)	1.00	188 (36)	55 (43)	88 (40)	0.19
Methotrexate	5 (1)	0 (0)	3 (2)	—	1 (1)	0 (0)	1 (1)	—	6 (1)	0 (0)	4 (2)	—
Antibiotics	22 (6)	5 (8)	13 (10)	0.78	15 (9)	6 (10)	10 (11)	1.00	37 (7)	11 (9)	23 (10)	0.71
CRP—no. (%)												
$\geqslant$25% decrease in CRP within 22 weeks. Baseline CRP $\geqslant 20\,mg\,l^{-1}$	66 (19)	7 (11)	20 (16)	0.16	30 (18)	6 (10)	9 (10)	0.15	96 (19)	13 (10)	29 (13)	0.02

Abbreviations: CD, Crohn's disease; CRP, C-reactive protein; IBD, inflammatory bowel disease; UC, ulcerative colitis.

Table 2. The biological effect of the studied SNP, OR and association with anti-TNF therapy among patients with CD, UC and combined IBD

Gene (SNP)	*rs number*	*Effect of the SNP*	*OR (95% CI)*	*Association*
TLR2 *(activates inflammation through the canonical NFκB pathway)*				
A>T	rs4696480	Unknown[38]	0.47 (0.23–0.95)[c]	Nonresponse (TT)[c]
C>A	rs11938228	Unknown[38]	0.63 (0.41–0.98)[a]	Nonresponse (CA or AA)[a,c]
C>T	rs1816702	rs1816702T increases receptor level[e,16]	2.02 (1.04–3.95)[b]	Response (CT or TT)[b]
597T>C	rs3804099	597C decreases TNF-α, IL-1β and IL-6 levels[g,36]	1.80 (1.15–2.81)[a]	Response (TC or CC)[a,b,c]
TLR4 *(activates inflammation through the canonical or noncanonical NFκB pathway)*				
G>A	rs5030728	Unknown[38]	1.45 (1.06–2.00)[a]	Response (GA or AA)[a,b,c]
T>C	rs1554973	Unknown[38]	0.72 (0.52–0.99)[a]	Nonresponse (TC or CC)[a]
T>C	rs12377632	Unknown[38]	—	No association
TLR5 *(activates inflammation through the canonical NFκB pathway)*				
1174C>T	rs5744168	1174T (392^{STOP}) decreases TNF-α, IL-1β and IL-6 level[g,36] and inhibits TLR5 function[f,g,39]	—	No association
TLR9 *(activates inflammation through the canonical NFκB pathway)*				
−1486T>C	rs187084	−1486C and 1174G decrease expression[f,19]	1.99 (1.04–3.82)[a]	Response (TC)[a]
1174G>A	rs352139	−1486C and 1174G decrease expression[f,19]	0.48 (0.24–0.96)[a]	Nonresponse (AA)[a,b]
LY96 *(MD-2 binds to TLR2 or TLR4 and is required for their activation to LPS stimuli)*				
−1625C>G	rs11465996	−1625G increases MD-2 and TNF-α levels[f,g,15]	1.48 (1.00–2.19)[a]	Response (CG or GG)[a,b,c,d]
CD14 *(binds LPS and transport it to TLR4)*				
−159G>A	rs2569190	−159AA increases CD14 level[g,17,18]	0.54 (0.30–0.98)[c]	Nonresponse (GA or AA)[c]
MAP3K14 *(NIK is a central kinase in the noncanonical NFκB pathway)*				
T>C	rs7222094	rs7222094CC decreases NIK activity[g,21]	1.92 (1.00–3.68)[a]	Response (TC)[a]
SUMO4 *(SUMO4 conjugates to IκBα and negatively regulates NFκB transcriptional activity)*				
163T>C	rs237025	163C increases NFκB1 expression[f,40]	—	No association
NFKBIA *(IkBα is an inhibitor of NFκB1)*				
2758A>G	rs696	2758A increases expression[f,41]	—	No association
T>del	rs17103265	rs17103265del decreases expression[f,42]	—	No association
NFKB1 *(NFκB1 (p50-RelA) is a transcription factor)*				
−94 ins per del	rs28362491	−94del decreases expression[f,i,43]	—	No association
TNFA *(TNF-α is a pro-inflammatory cytokine activated by NFκB1)*				
−863C>A	rs1800630	−863A increases expression[f,i,44]	—	Failed to genotype
−857C>T	rs1799724	−857T increases TNF-α level[f,g,h,45]	—	Failed to genotype
−308G>A	rs1800629	−308A increases expression[e,f,46]	—	No association
−238G>A	rs361525	−238A decreases expression[f,g,25]	0.43 (0.19–0.97)[a]	Nonresponse (GA)[a]
TNFRSF1A *(TNF receptor 1 (TNFR1) binds TNF-α and initiates a kinase cascade)*				
−609G>T	rs4149570	−609T increases expression[h,47]	2.07 (1.03–4.15)[a]	Response (TT)[a,b]
TNFAIP3 *(TNF-α rapidly induced expression of TNFAIP3 (A20) that inhibit NFκB activation and TNF-α mediated apoptosis)*				
C>G	rs6927172	rs6927172G increases expression[f,i,27]	0.62 (0.42–0.92)[a]	Nonresponse (CG or GG)[a,c]
IL1B *(pro-inflammatory cytokine activated by NFκB1)*				
−3737G>A	rs4848306	−3737A decreases transcription[h,26,29]	1.85 (1.05–3.27)[a]	Response (GA or AA)[a,c]
−1464G>C	rs1143623	rs1143623C decreases IL-1β level[g,26,32]	—	No association
−31T>C	rs1143627	−31C decreases expression[f,g,i,26,31,32]	—	No association
IL1RN *(IL-1RA binds to the IL-1 receptor and inhibits IL-1β signaling)*				
T>C	rs4251961	rs4251961C decreases IL-1RA level[g,33,48]	0.42 (0.18–0.98)[c]	Nonresponse (TC or CC)[c]
IL4R *(IL-4 receptor, IL-4 significantly inhibits IL-17 production)*				
A>G (I50V)	rs1805010	rs1805010G increases IL-17 level[e,g,49]	—	No association

Table 2. (Continued)

Gene (SNP)	*rs number*	*Effect of the SNP*	*OR (95% CI)*	*Association*
IL6 *(pro- and anti-inflammatory cytokine activated by NFκB1)*				
–6331T>C	rs10499563	–6331C decreases expression[f,i,30]	2.26 (1.18–4.32)[a]	Response (TC or CC)[a,c]
IL6R *(binds IL-6 and initiates a kinase cascade)*				
C>T	rs4537545	rs4537545TT increases IL-6r and IL-6 levels but not TNF-α, IL-1RA and CRP levels[g,34]	—	No association
IL10 *(activated by NFκB1, capable of inhibiting synthesis of pro-inflammatory cytokines such as IFN-γ and TNF-α)*				
–592 C>A	rs1800872	–592A increases expression[f,50]	—	No association
C>T	rs3024505	Unknown. Associated with IBD[51]	—	No association
IL17A *(activated by NFκB1, pro-inflammatory, potent mediator in delayed-type reactions, induces production of IL-1β, IL-6 and TNF-α)*				
197G>A	rs2275913	197A increases expression[f,h,i,37]	0.42 (0.18–1.00)[c]	Nonresponse (GA or AA)[a,c]
IL23R *(IL-23 receptor, IL-23 induces the production of IL-17 and IFN-γ)*				
G>A (R381Q)	rs11209026	rs11209026GG increases IL-17 serum level[g,52]	—	No association
IFNG *(IFN-γ is a pro- and anti-inflammatory cytokine activated by NFκB1)*				
874T>A	rs2430561	874A decreases IFN-γ level[g,28]	1.66 (1.05–2.62)[a]	Response (TA or AA)[a,b]
TGFB1 *(TGF-β1 is a cytokine that can inhibit the secretion and activity of many other cytokines including IFN-γ and TNF-α)*				
–509C>T	rs1800469	–509T increases expression[f,i,53]	—	No association
PTPN22 *(involved in several signaling pathways associated with the immune response)*				
1858G>A	rs2476601	1858A decreases TNF-α in serum[g,h,54]	—	No association
PPARG *(PPARγ is a transcription factor)*				
C>G	rs1801282	rs1801282G decreases PPARγ mRNA level, but upregulates MyD88 TLR4, TLR5, TLR9, P65 and TNF-α mRNA levels[g,h,55]	—	No association
NLRP3 *(NALP3 is involved in the inflammasome)*				
C>T	rs4612666	rs4612666T decreases expression[f,56]	—	No association

Abbreviations: CD, Crohn's disease; CI, confidence interval; CRP, C-reactive protein; IBD, inflammatory bowel disease; LPS, lipopolysaccharide; OR, odds ratios; SNP, single nucleotide polymorphism; UC, ulcerative colitis. [a]Association among patients with IBD. [b]Association among patients with CD. [c]Association among patients with UC. [d]The polymorphism showed opposite direction of effect among patients with CD and UC. [e]Function examined by flow cytometry. [f]Function examined by luciferase reporter assay. [g]Function examined by enzyme-linked immunosorbent assay. [h]Function examined by reverse transcriptase PCR. [i]Function examined by electrophoretic mobility shift assay.

(*IL1RN* T>C (rs4251961)), decrease *IL6* expression (*IL6* –6331T>C (rs10499563)) and increase *IL17A* expression (*IL17A* 197G>A (rs2275913)) (Table 2). The biological effect of the polymorphisms *TLR2* A>T (rs4696480), *TLR2* C>A (rs11938228) and *TLR4* G>A (rs5030728) was unknown.

Thus, genetically determined increased MD-2 level (rs11465996), CD14 level (rs11465996), TNF-α level (rs11465996), *TNFAIP3* (A20) expression (rs6927172) and IL-17 expression (rs2275913) were associated with nonresponse and genetically determined decreased *IL1B* (rs4848306 and rs3804099) and *IL6* expressions (rs10499563 and rs3804099) were associated with beneficial response among patients with UC. One polymorphism indicated that decreased TNF-α level (along with decreased IL-1β and IL-6 levels) (rs3804099) was associated with beneficial response. Finally, genetically determined decreased IL-1RA level (rs4251961) was associated with nonresponse.

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

The polymorphisms generally showed the same direction of effect in both diseases, except for the polymorphisms in *LY96* (rs11465996) (Supplementary Tables 1 and 2).

In a dominant model, the homozygous variant genotype of *TNFRSF1A* –609G>T (rs4149570) (OR_{adj}: 2.07, 95% CI: 1.03–4.15, $P=0.04$), the heterozygous genotypes of *TLR9* –1486T>C (rs187084) (OR_{adj}: 1.99, 95% CI: 1.04–3.82, $P=0.04$) and *MAP3K14* T>C (rs7222094) (OR_{adj}: 1.92, 95% CI: 1.00–3.68, $P=0.05$) and both the homozygous and the heterozygous variant genotypes of *TLR2* 597T>C (rs3804099) (OR_{adj}: 1.80, 95% CI: 1.15–2.81, $P=0.01$), *TLR4* G>A (rs5030728) (OR_{unadj}: 1.45, 95% CI: 1.06–2.00, $P=0.02$), *LY96* (MD-2) –1625 C>G (rs11465996) (OR_{unadj}: 1.48, 95% CI: 1.00–2.19, $P=0.05$), *IL1B* –3737G>A (rs4848306) (OR_{adj}: 1.85, 95% CI: 1.05–3.27, $P=0.03$), *IL6* –6331T>C (rs10499563) (OR_{adj}: 2.26, 95% CI: 1.18–4.32, $P=0.01$) and *IFNG* 874T>A (rs2430561) (OR_{adj}: 1.66, 95% CI: 1.05–2.62, $P=0.03$) were associated with beneficial response among patients with IBD. The heterozygous genotype of *TNFA* –238G>A (rs361525) (OR_{adj}: 0.43, 95% CI: 0.19–0.97, $P=0.04$) and both the homozygous and the heterozygous variant genotypes of *TLR2* C>A (rs11938228) (OR_{adj}: 0.63, 95% CI: 0.41–0.98, $P=0.04$), *TLR4* T>C (rs1554973) (OR_{unadj}: 0.72, 95% CI: 0.52–0.99, $P=0.04$) and *TNFAIP3* (A20) C>G (rs6927172) (OR_{adj}: 0.62, 95% CI: 0.42–0.92, $P=0.02$) were associated with nonresponse (Supplementary Table 3).

In a recessive model, the homozygous variant genotype of *TLR2* 597T>C (rs3804099) (OR_{adj}: 2.52, 95% CI: 1.08–5.87, $P=0.03$) and the homozygous variant genotype of *TNFRSF1A* –609G>T (rs4149570) (OR_{adj}: 2.65, 95% CI: 1.00–6.97, $P=0.05$) were associated with beneficial response. The homozygous variant genotype of *TLR9* 1174G>A (rs352139) (OR_{adj}: 0.48, 95% CI: 0.24–0.96, $P=0.04$) and the homozygous variant genotype of *IL17A* 197G>A (rs2275913) (OR_{adj}: 0.47, 95% CI: 0.21–1.01, $P=0.05$) were associated with nonresponse (Supplementary Table 3).

The variant allele of the polymorphisms has been shown to decrease TNF-α, IL-1β and IL-6 levels (*TLR2* 597T>C (rs3804099)), increase MD-2 and TNF-α levels (*LY96* −1625 C>G (rs11465996)), decrease *TNFA* expression (*TNFA* −238G>A (rs361525)), increase *TNFRSF1A* (*TNFRSF1A* −609G>T (rs4149570)), *TNFAIP3* expressions (*TNFAIP3* C>G (rs6927172)), decrease *IL1B* transcription (*IL1B* −3737G>A (rs4848306)), decrease *IL6* expression (*IL6* −6331T>C (rs10499563)), increase *IL17A* expression (*IL17A* 197G>A (rs2275913)) and decrease IFN-γ level (*IFNG* 874T>A (rs2430561)) (Table 2). The polymorphisms −1486T>C (rs187084) and 1174G>A (rs352139) in *TLR9* have only been shown to have a biological effect in a haplotype context and the biological effect of the heterozygous variant of *MAP3K14* T>C (rs7222094) was unknown. The biological effect of the polymorphisms *TLR2* C>A (rs11938228), *TLR4* G>A (rs5030728) and *TLR4* T>C (rs1554973) was unknown.

Thus, genetically determined increased MD-2 level (rs11465996) and *TNFRSF1A* (TNFR1) expression (rs4149570) and genetically determined decreased *TNFAIP3* (A20) expression (rs6927172), IL-1β (rs3804099 and rs4848306), IL-6 (rs3804099 and rs10499563), IL-17 (rs2275913) and IFN-γ (rs2430561) levels were associated with beneficial response among patients with IBD. Again, the effect of TNF-α was less clear, as two polymorphisms indicated that genetically determined increased TNF-α level (rs11465996 and rs361525) and one polymorphism indicated that decreased TNF-α level (rs3804099) (along with decreased IL-1β and IL-6 levels) were associated with beneficial response.

Haplotype analysis

Haplotype analysis of *TLR2*, *TLR4*, *TLR9* and *IL1B* among patients with IBD is shown in Supplementary Tables 4–7, respectively. Rs11938228 and rs3804099 in *TLR2* were in linkage disequilibrium with $r^2 = 0.34$ and $D' = 1.00$. In *TLR9*, the linkage disequilibrium for rs187084 and rs352139 was $r^2 = 0.55$ and $D' = 1.00$.

Four haplotypes in *TLR2*, three in *TLR4* and *IL1B* and two in *TLR9* described 85, 95, 99 and 97% of the genotypes observed, respectively. The *TLR2* haplotype 22 (rs4696480TT, rs1816702CC, rs11938228AA and rs3804099TT) (OR: 0.41, 95% CI: 0.19–0.86, $P = 0.02$) and the haplotype 12 (rs4696480TA, rs1816702CC, rs11938228CA and rs3804099CT) (OR: 0.48, 95% CI: 0.24–0.95, $P = 0.04$) were associated with nonresponse. Haplotype combination 33 was also associated with nonresponse, although not statistically significant. Both haplotypes 2 and 3 encompass the wild-type allele of rs3804099, and thus the haplotype analysis supports the found association between the variant allele of rs3804099 and beneficial response. No associations were found for *TLR4,TLR9* or *IL1B*.

DISCUSSION

In the inflammatory pathways, 37 SNPs in 26 genes were successfully genotyped and 19 of the functional polymorphisms in 14 genes were associated with response to anti-TNF therapy among patients with CD, UC, or CD and UC combined (IBD) as shown in Figure 2.

As illustrated in Figure 1, genetically determined increased levels of TLR2 (rs1816702) and MD-2 (*LY96*) (rs11465996) (required for TLR2 and TLR4 to respond to LPS)[15,16] were associated with beneficial response among patients with CD, indicating that a higher activity of TLR2 was associated with a beneficial response among patients with CD. Among patients with UC, genetically determined increased levels of MD-2 (*LY96*) (rs11465996) and CD14 (rs2569190)[15,17,18] were associated with nonresponse, indicating that a high activity of TLR4 was associated with nonresponse among patients with UC. In addition, two SNPs in *TLR2* (rs4696480 and rs11938228) and two SNPs in *TLR4* (rs5030728 and rs1554973) with unknown biological effects were associated with response among patients with CD, UC or IBD as shown in Table 2. The *TLR9* heterozygous genotype of −1486T>C (rs187084) and the homozygous variant genotype of *TLR9* 1174G>A (rs352139) were associated with beneficial response and nonresponse among patients with IBD, respectively. The 1486T>C and 1174G>A polymorphisms in *TLR9* have only been shown to have a biological effect in haplotype context,[19,20] however, the haplotype analysis of *TLR9* did not reveal any associations. Thus, the results indicate that TLR activity is important in determining response to anti-TNF therapy among patients with IBD.

Regarding the canonical and noncanonical NFκB pathway, functional polymorphisms in *SUMO4*, *NFKBIA* (IκBα) and *NFKB1* (p50-RelA) were not found to be associated with response. However, the heterozygous genotype of rs7222094 T>C in *MAP3K14* (NIK) was associated with beneficial response among patients with IBD. The biological effect of the heterozygous genotype is unknown,[21] which makes it difficult to interpret the association in *MAP3K14* from a biological perspective. Further studies of the noncanonical NFκB pathway, for example, by studying functional polymorphisms in *LTA* or *TNFSF11* (RANKL),[22–24] could shed more light on any possible involvement of this pathway in anti-TNF therapy response.

The TNF-α signaling pathway showed that a genetically determined decreased expression of *TNFA* (TNF-α) (rs361525)[25] was associated with nonresponse among patients with IBD. Furthermore, a genetically determined increased expression of the TNF receptor 1 (*TNFRSF1A*) (rs4149570)[26] was associated with beneficial response among patients with CD and IBD. In addition, a genetically determined increased expression of *TNFAIP3* (A20) (rs6927172)[27] was associated with nonresponse among patients with IBD. A20, encoded by *TNFAIP3*, is known to inhibit NFκB activation as well as TNF-α-mediated apoptosis.

Thus, the results indicate that polymorphisms in *TNFA* (TNF-α), *TNFRSF1A* (TNFR1) and *TNFAIP3* (A20), which upregulate TNF-α

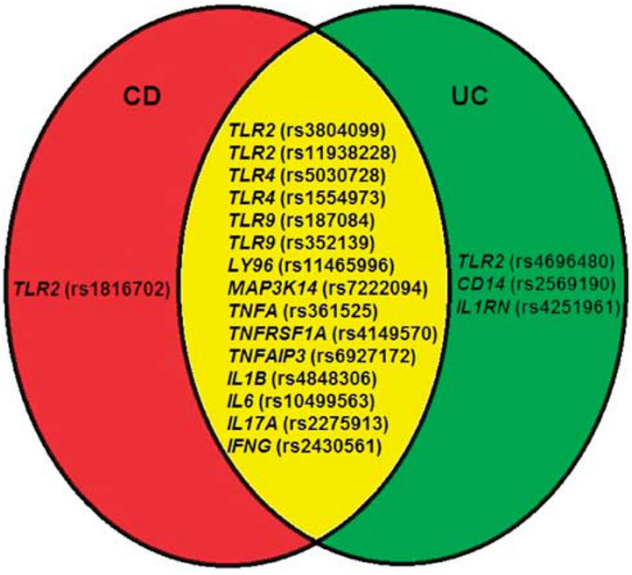

Figure 2. Polymorphisms associated with response to anti-TNF. Thirty-seven functional single nucleotide polymorphisms (SNPs) in 26 genes were successfully genotyped and 19 SNPs in 14 genes were associated with response to antitumor necrosis factor-α (TNF-α) therapy among patients with Crohn's disease (CD), ulcerative colitis (UC) or CD and UC combined. The 19 SNPs associated with response were in genes involved in regulation of NFκB through the Toll-like receptor (TLR) pathways (*TLR2*, *TLR4*, *TLR9*, *LY96* (MD-2), *CD14* and *MAP3K14* (NIK)), TNF-α signaling (*TNFA* (TNF-α), *TNFRSF1A* (TNFR1) and *TNFAIP3* (A20)) or cytokines regulated by NFκB (*IL1B*, *IL1RN*, *IL6*, *IL17A* and *IFNG*).

signaling, were associated with beneficial response to anti-TNF therapy among patients with IBD.

Among cytokines regulated by NFκB, a genetically determined decreased expression of *IL1B* (rs4848306), *IL6* (rs10499563) and *IFNG* (rs2430561)[28–30] were associated with beneficial response among patients with CD, UC and IBD. Furthermore, a polymorphism in *IL1B* (rs1143627), which has been shown to increase IL-1β level,[31,32] was borderline significantly associated with nonresponse among patients with IBD. In addition, a genetically determined decreased IL-1 receptor antagonist (IL-1RA) level (rs4251961)[33] was associated with nonresponse among patients with UC. IL-1RA binds to the IL-1 receptor and inhibits IL-1β signaling.[3] The IL-6 receptor is not regulated by NFκB and no association was found with the SNP studied in *IL6R*.[34]

This could indicate that among patients with IBD, nonresponders to anti-TNF therapy are more likely to have an inflammatory response mediated by other early pro-inflammatory cytokines than TNF-α, such as IL-1β, IL-6 and IFN-γ. This suggests that drugs targeting IFN-γ, IL-1β or IL-6 could potentially be useful for treating patients who do not respond to anti-TNF therapy. This interpretation is supported by the polymorphism in *IL1RN,* where a genetically determined high inhibition of IL-1β signaling (high IL-1RA level) was associated with beneficial response. Furthermore, this interpretation is also supported by another study that has reported an association between the C-allele of rs1143634 in *IL1B* and higher serum IL-1β level and a lower response rate to infliximab therapy among patients with CD.[35]

The variant allele of the 597T>C polymorphism in *TLR2* (rs3804099) was associated with beneficial response among patients with CD, UC and IBD. The polymorphism has been shown to decrease TNF-α level by ~50%, IL-1β level by 75% and IL-6 level by 150%.[36] In the light of the other results, we expected an increased TNF-α level to be associated with beneficial response and an increased IL-1β and IL-6 levels to be associated with nonresponse. This indicates that the relative levels of the cytokines TNF-α, IL-1β and IL-6 are important in determining response to anti-TNF.

The variant allele of the 197G>A polymorphism in *IL17A* was associated with nonresponse among patients with UC and IBD. The polymorphism has been shown to increase expression of IL-17,[37] indicating that high level of this cytokine may also be associated with nonresponse.

The functional SNPs studied in *IL4R*, *IL10*, *IL23R*, *TGFB1*, *PTPN22*, *PPARG* and *NLRP3* were not associated with response to anti-TNF therapy.

Overall, the results indicate that patients with genetically determined high TNF-driven inflammatory response benefit the most from anti-TNF therapy. Conversely, patients with genetically determined IL1B, IL6 and IFNG-driven inflammatory response seem to benefit the least from anti-TNF therapy. These patients might benefit from biological drugs targeting other cytokines such as IL-1β, IL-6 or IFN-γ or from a cocktail of several antibodies.

The results in this exploratory study should be interpreted with care. Additional confirmation of these findings in independent cohorts should be performed before our results are applied in the clinic. 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. We successfully genotyped 37 polymorphisms. Of these, two polymorphisms would be expected to be associated with treatment outcome by pure chance assuming a 5% acceptance level. We found numerous associated polymorphisms. Furthermore, most of the found associations were biologically plausible. The study generally did not have enough power to detect a recessive effect and the associations were predominantly found to show gene-dose effects. We cannot exclude that associations were not identified due to insufficient statistical power. On the other hand, this study is rather large including 738 IBD patients treated with anti-TNF, giving the cohort study >80% power to detect an OR of 1.6 assuming a minor allele frequency of 0.05. In addition, blood and clinical data from these patients were collected at 18 large gastroenterological centers at basic and specialized hospitals in Denmark. Thus, the patients are representative of Danish patients with severe IBD.

In conclusion, the results suggest that genes involved in the regulation of NFκB through the TLR pathways, genes regulating TNF-α signaling and cytokines regulated by NFκB are important predictors for the response to anti-TNF therapy among patients with IBD. Genetically strong TNF-mediated inflammatory response was associated with beneficial response to anti-TNF therapy. In addition, patients with genetically determined high IL-1β, IL-6 or IFN-γ levels were less likely to respond, perhaps because the colonic inflammation was primarily driven by these pro-inflammatory cytokines. This could indicate that the cytokines IL-1β, IL-6 and IFN-γ may be potential targets for treating patients with IBD who do not respond to anti-TNF therapy. Before the genetic markers found in this study are applied in a clinical setting, they should be confirmed in independent cohorts.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The work was funded by Health Research Fund of Central Denmark Region, Colitis-Crohn Foreningen and University of Aarhus (PhD grant).

REFERENCES

1 Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417–429.
2 http://www.bu.edu/nf-kb/gene-resources/target-genes/.
3 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. *Cell Mol Life Sci* 2008; **65**: 2964–2978.
4 Komatsu M, Kobayashi D, Saito K, Furuya D, Yagihashi A, Araake H *et al.* Tumor necrosis factor-alpha in serum of patients with inflammatory bowel disease as measured by a highly sensitive immuno-PCR. *Clin Chem* 2001; **47**: 1297–1301.
5 Dionne S, Hiscott J, D'Agata I, Duhaime A, Seidman EG. Quantitative PCR analysis of TNF-alpha and IL-1 beta mRNA levels in pediatric IBD mucosal biopsies. *Dig Dis Sci* 1997; **42**: 1557–1566.
6 Meena NK, Verma R, Verma N, Ahuja V, Paul J. TLR4 D299G polymorphism modulates cytokine expression in ulcerative colitis. *J Clin Gastroenterol* 2013; **47**: 773–780.
7 Knight DM, Trinh H, Le J, Siegel S, Shealy D, McDonough M *et al.* Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. *Mol Immunol* 1993; **30**: 1443–1453.
8 Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J *et al.* Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462–2476.
9 Mascheretti S, Hampe J, Kuhbacher T, Herfarth H, Krawczak M, Folsch UR *et al.* Pharmacogenetic investigation of the TNF/TNF-receptor system in patients with chronic active Crohn's disease treated with infliximab. *Pharmacogenomics J* 2002; **2**: 127–136.
10 Ljung T, Karlen P, Schmidt D, Hellstrom PM, Lapidus A, Janczewska I *et al.* Infliximab in inflammatory bowel disease: clinical outcome in a population based cohort from Stockholm County. *Gut* 2004; **53**: 849–853.
11 Caspersen S, Elkjaer M, Riis L, Pedersen N, Mortensen C, Jess T *et al.* Infliximab for inflammatory bowel disease in Denmark 1999-2005: clinical outcome and follow-up evaluation of malignancy and mortality. *Clin Gastroenterol Hepatol* 2008; **6**: 1212–1217.
12 Cohen RD, Tsang JF, Hanauer SB. Infliximab in Crohn's disease: first anniversary clinical experience. *Am J Gastroenterol* 2000; **95**: 3469–3477.
13 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. *Genet Test Mol Biomarkers* 2013; **17**: 501–503.
14 http://pngu.mgh.harvard.edu/~purcell/gpc/.
15 Gu W, Shan YA, Zhou J, Jiang DP, Zhang L, Du DY *et al.* Functional significance of gene polymorphisms in the promoter of myeloid differentiation-2. *Ann Surg* 2007; **246**: 151–158.

16 Bielinski SJ, Hall JL, Pankow JS, Boerwinkle E, Matijevic-Aleksic N, He M *et al*. Genetic variants in TLR2 and TLR4 are associated with markers of monocyte activation: the Atherosclerosis Risk in Communities MRI Study. *Hum Genet* 2011; **129**: 655–662.
17 Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5′ flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999; **20**: 976–983.
18 Mertens J, Bregadze R, Mansur A, Askar E, Bickeboller H, Ramadori G *et al*. Functional impact of endotoxin receptor CD14 polymorphisms on transcriptional activity. *J Mol Med (Berl)* 2009; **87**: 815–824.
19 Tao K, Fujii M, Tsukumo S, Maekawa Y, Kishihara K, Kimoto Y *et al*. Genetic variations of Toll-like receptor 9 predispose to systemic lupus erythematosus in Japanese population. *Ann Rheum Dis* 2007; **66**: 905–909.
20 Omar AH, Yasunami M, Yamazaki A, Shibata H, Ofori MF, Akanmori BD *et al*. Toll-like receptor 9 (TLR9) polymorphism associated with symptomatic malaria: a cohort study. *Malar J* 2012; **11**: 168.
21 Thair SA, Walley KR, Nakada TA, McConechy MK, Boyd JH, Wellman H *et al*. A single nucleotide polymorphism in NF-kappaB inducing kinase is associated with mortality in septic shock. *J Immunol* 2011; **186**: 2321–2328.
22 Knight JC, Keating BJ, Kwiatkowski DP. Allele-specific repression of lymphotoxin-alpha by activated B cell factor-1. *Nat Genet* 2004; **36**: 394–399.
23 Migita O, Noguchi E, Koga M, Jian Z, Shibasaki M, Migita T *et al*. Haplotype analysis of a 100 kb region spanning TNF-LTA identifies a polymorphism in the LTA promoter region that is associated with atopic asthma susceptibility in Japan. *Clin Exp Allergy* 2005; **35**: 790–796.
24 Tan W, Wu H, Zhao J, Derber LA, Lee DM, Shadick NA *et al*. A functional RANKL polymorphism associated with younger age at onset of rheumatoid arthritis. *Arthritis Rheum* 2010; **62**: 2864–2875.
25 Kaluza W, Reuss E, Grossmann S, Hug R, Schopf RE, Galle PR *et al*. Different transcriptional activity and in vitro TNF-alpha production in psoriasis patients carrying the TNF-alpha 238A promoter polymorphism. *J Invest Dermatol* 2000; **114**: 1180–1183.
26 Chen H, Wilkins LM, Aziz N, Cannings C, Wyllie DH, Bingle C *et al*. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. *Hum Mol Genet* 2006; **15**: 519–529.
27 Elsby LM, Orozco G, Denton J, Worthington J, Ray DW, Donn RP. Functional evaluation of TNFAIP3 (A20) in rheumatoid arthritis. *Clin Exp Rheumatol* 2010; **28**: 708–714.
28 Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. *Hum Immunol* 2000; **61**: 863–866.
29 Yoshida M, Shiroiwa K, Mouri K, Ishiguro H, Supriyanto I, Ratta-Apha W *et al*. Haplotypes in the expression quantitative trait locus of interleukin-1beta gene are associated with schizophrenia. *Schizophr Res* 2012; **140**: 185–191.
30 Smith AJ, D'Aiuto F, Palmen J, Cooper JA, Samuel J, Thompson S *et al*. Association of serum interleukin-6 concentration with a functional IL6 -6331T>C polymorphism. *Clin Chem* 2008; **54**: 841–850.
31 Lind H, Haugen A, Zienolddiny S. Differential binding of proteins to the IL1B -31 T/C polymorphism in lung epithelial cells. *Cytokine* 2007; **38**: 43–48.
32 Wen AQ, Gu W, Wang J, Feng K, Qin L, Ying C *et al*. Clinical relevance of IL-1beta promoter polymorphisms (-1470, -511, and -31) in patients with major trauma. *Shock* 2010; **33**: 576–582.
33 Rafiq S, Stevens K, Hurst AJ, Murray A, Henley W, Weedon MN *et al*. Common genetic variation in the gene encoding interleukin-1-receptor antagonist (IL-1RA) is associated with altered circulating IL-1RA levels. *Genes Immun* 2007; **8**: 344–351.
34 Rafiq S, Frayling TM, Murray A, Hurst A, Stevens K, Weedon MN *et al*. A common variant of the interleukin 6 receptor (IL-6r) gene increases IL-6r and IL-6 levels, without other inflammatory effects. *Genes Immun* 2007; **8**: 552–559.
35 Lacruz-Guzman D, Torres-Moreno D, Pedrero F, Romero-Cara P, Garcia-Tercero I, Trujillo-Santos J *et al*. Influence of polymorphisms and TNF and IL1beta serum concentration on the infliximab response in Crohn's disease and ulcerative colitis. *Eur J Clin Pharmacol* 2013; **69**: 431–438.
36 Zhang F, Gao XD, Wu WW, Gao Y, Zhang YW, Wang SP. Polymorphisms in toll-like receptors 2, 4 and 5 are associated with Legionella pneumophila infection. *Infection* 2013; **41**: 941–948.
37 Espinoza JL, Takami A, Nakata K, Onizuka M, Kawase T, Akiyama H *et al*. A genetic variant in the IL-17 promoter is functionally associated with acute graft-versus-host disease after unrelated bone marrow transplantation. *PLoS One* 2011; **6**: e26229.
38 Gast A, Bermejo JL, Claus R, Brandt A, Weires M, Weber A *et al*. Association of inherited variation in Toll-like receptor genes with malignant melanoma susceptibility and survival. *PLoS One* 2011; **6**: e24370.
39 Hawn TR, Verbon A, Lettinga KD, Zhao LP, Li SS, Laws RJ *et al*. A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. *J Exp Med* 2003; **198**: 1563–1572.
40 Guo D, Li M, Zhang Y, Yang P, Eckenrode S, Hopkins D *et al*. A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. *Nat Genet* 2004; **36**: 837–841.
41 Song S, Chen D, Lu J, Liao J, Luo Y, Yang Z *et al*. NFkappaB1 and NFkappaBIA polymorphisms are associated with increased risk for sporadic colorectal cancer in a southern Chinese population. *PLoS One* 2011; **6**: e21726.
42 Wang S, Zhang M, Zeng Z, Tian L, Wu K, Chu J *et al*. IkappaBalpha polymorphisms were associated with increased risk of gastric cancer in a southern Chinese population: a case-control study. *Life Sci* 2011; **88**: 792–797.
43 Park JY, Farrance IK, Fenty NM, Hagberg JM, Roth SM, Mosser DM *et al*. NFKB1 promoter variation implicates shear-induced NOS3 gene expression and endothelial function in prehypertensives and stage I hypertensives. *Am J Physiol Heart Circ Physiol* 2007; **293**: H2320–H2327.
44 Udalova IA, Richardson A, Denys A, Smith C, Ackerman H, Foxwell B *et al*. Functional consequences of a polymorphism affecting NF-kappaB p50-p50 binding to the TNF promoter region. *Mol Cell Biol* 2000; **20**: 9113–9119.
45 Lv K, Chen R, Cai Q, Fang M, Sun S. Effects of a single nucleotide polymorphism on the expression of human tumor necrosis factor-alpha. *Scand J Immunol* 2006; **64**: 164–169.
46 Karimi M, Goldie LC, Cruickshank MN, Moses EK, Abraham LJ. A critical assessment of the factors affecting reporter gene assays for promoter SNP function: a reassessment of -308 TNF polymorphism function using a novel integrated reporter system. *Eur J Hum Genet* 2009; **17**: 1454–1462.
47 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. *Hum Immunol* 2011; **72**: 451–457.
48 Carrol ED, Payton A, Payne D, Miyajima F, Chaponda M, Mankhambo LA *et al*. The IL1RN promoter rs4251961 correlates with IL-1 receptor antagonist concentrations in human infection and is differentially regulated by GATA-1. *J Immunol* 2011; **186**: 2329–2335.
49 Wallis SK, Cooney LA, Endres JL, Lee MJ, Ryu J, Somers EC *et al*. A polymorphism in the interleukin-4 receptor affects the ability of interleukin-4 to regulate Th17 cells: a possible immunoregulatory mechanism for genetic control of the severity of rheumatoid arthritis. *Arthritis Res Ther* 2011; **13**: R15.
50 Rees LE, Wood NA, Gillespie KM, Lai KN, Gaston K, Mathieson PW. The interleukin-10-1082 G/A polymorphism: allele frequency in different populations and functional significance. *Cell Mol Life Sci* 2002; **59**: 560–569.
51 Andersen V, Ernst A, Christensen J, Ostergaard M, Jacobsen BA, Tjonneland A *et al*. The polymorphism rs3024505 proximal to IL-10 is associated with risk of ulcerative colitis and Crohns disease in a Danish case-control study. *BMC Med Genet* 2010; **11**: 82.
52 Oosting M, ter HH, van de Veerdonk FL, Sturm P, Kullberg BJ, van der Meer JW *et al*. Role of interleukin-23 (IL-23) receptor signaling for IL-17 responses in human Lyme disease. *Infect Immun* 2011; **79**: 4681–4687.
53 Shah R, Hurley CK, Posch PE. A molecular mechanism for the differential regulation of TGF-beta1 expression due to the common SNP -509C-T (c. -1347C > T). *Hum Genet* 2006; **120**: 461–469.
54 Kariuki SN, Crow MK, Niewold TB. The PTPN22 C1858T polymorphism is associated with skewing of cytokine profiles toward high interferon-alpha activity and low tumor necrosis factor alpha levels in patients with lupus. *Arthritis Rheum* 2008; **58**: 2818–2823.
55 Aoyagi Y, Nagata S, Kudo T, Fujii T, Wada M, Chiba Y *et al*. Peroxisome proliferator-activated receptor gamma 2 mutation may cause a subset of ulcerative colitis. *Pediatr Int* 2010; **52**: 729–734.
56 Hitomi Y, Ebisawa M, Tomikawa M, Imai T, Komata T, Hirota T *et al*. Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma. *J Allergy Clin Immunol* 2009; **124**: 779–785.

Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)