The Pharmacogenomics Journal (2018) 18, 494–500
www.nature.com/tpj

OPEN

ORIGINAL ARTICLE

Associations between functional polymorphisms and response to biological treatment in Danish patients with psoriasis

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Biological agents including anti-tumor necrosis factor (anti-TNF; adalimumab, infliximab, etanercept) and anti-interleukin-12/13 (IL12/23; ustekinumab) are essential for treatment of patients with severe psoriasis. However, a significant proportion of the patients do not respond to a specific treatment. Pharmacogenetics might be a way to predict treatment response. Using a candidate gene approach, 62 mainly functional single-nucleotide polymorphisms (SNPs) in 44 different genes were evaluated in 478 Danish patients with psoriasis undergoing 376 series of anti-TNF treatment and 230 series of ustekinumab treatment. Associations between genetic variants and treatment outcomes (drug survival and Psoriasis Area Severity Index reduction) were assessed using logistic regression analyses (crude and adjusted for gender, age, psoriatic arthritis and previous treatment). After correction for multiple testing controlling the false discovery rate, six SNPs (*IL1B* (rs1143623, rs1143627), *LY96* (rs11465996), *TLR2* (rs11938228, rs4696480) and *TLR9* (rs352139)) were associated with response to anti-TNF treatment and 4 SNPs (*IL1B* (rs1143623, rs1143627), *TIRAP* (rs8177374) and *TLR5* (rs5744174)) were associated with response to ustekinumab treatment ($q < 0.20$). The results suggest that genetic variants related to increased IL-1β levels may be unfavorable when treating psoriasis with either anti-TNF or ustekinumab, whereas genetic variants related to high interferon-γ levels may be favorable when treating psoriasis with ustekinumab.

The Pharmacogenomics Journal (2018) **18,** 494–500; doi:10.1038/tpj.2017.31; published online 11 July 2017

INTRODUCTION

Psoriasis is a common skin disease characterized by an inflamed, red scaly skin that affects 2–4% of the world's population.[1] Psoriasis involves a dysregulated immune system and is regarded as a T cell-mediated immune disease with a mixed upregulation of T helper type 1/17 (Th1/Th17) cytokines,[2] including tumor necrosis factor-α (TNF-α), interleukin (IL)-12, IL-23, IL-22, interferon-γ (IFN-γ) and IL-17A.[3] Biological drugs targeting TNF-α (infliximab adalimumab, etanercept) and the p40 subunit of IL12 and IL23 (ustekinumab) are essential for treatment of patients with severe psoriasis. However, approximately one-third of the patients do not respond to a specific treatment[4] and require a change in treatment. The consequences of this 'trial and error' policy results in periods of nonoptimal treatment for the patient and increased treatment costs, thus calling for ways of identifying the best treatment for each patient. Pharmacogenetics might be a way of predicting treatment response. However, little is known regarding the interaction between genetics and biological drugs in relation to psoriasis.[5] Pharmacogenetics has been more thoroughly investigated in inflammatory bowel disease and rheumatoid arthritis, where polymorphisms in genes encoding Toll-like receptors (TLRs) and NOD-like receptors have been found to be associated with response to anti-TNF drugs.[6–10] Nuclear factor-κB plays a major role in controlling inflammation and is an important regulator of pathways leading to expression of cytokines in psoriasis, including IL-1β (http://www.bu.edu/nf-kb/gene-resources/target-genes/). Nuclear factor-κB can be activated by TLRs and NOD-like receptors. The objective of this study was to determine whether variants in genes involved in nuclear factor-κB, TNF-α and pattern recognition (TLRs and NOD-like receptors) pathways could be used to predict response and nonresponse in patients with psoriasis who are treated either with anti-TNF or ustekinumab.

MATERIALS AND METHODS

Cohort

Blood clot samples, sent for *Mycobacterium tuberculosis* screening, were obtained from 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 Biochemistry, Hospital of Lillebaelt (Vejle, Denmark); the Department of Clinical Biochemistry, Herlev and Gentofte Hospital (Hellerup, Denmark); and the Department of Biochemistry, Hospital of Slagelse (Slagelse, Denmark) from September 2009 through July 2015 as described earlier.[6–9] Screening for *Mycobacterium tuberculosis* before initiation of treatment with biological drugs is generally performed in Denmark. Patients were identified by linking the unique personal identification number of Danish citizens (CPR number) from each blood

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Received 21 November 2016; revised 17 May 2017; accepted 26 May 2017; published online 11 July 2017

sample with the Danish national database DERMBIO (Figure 1). DERMBIO is a nationwide registry including all biological administered treatments for psoriasis in Denmark, with a 100% coverage of treatments administered in academic hospitals (88% of treatments) and an estimated coverage of >80% for private clinics (12% of treatments).[11] Prospective data were collected from DERMBIO and were supplemented with and validated by information from patient records. If a patient had been given more than one anti-TNF treatment, only the first treatment was included. If a patient had been treated with both anti-TNF and ustekinumab, then both treatment series were included (Figure 1). Patient data included gender; body mass index; age at baseline and at diagnosis; Psoriasis Area Severity Index (PASI)[12] and dermatology life quality index scores at baseline, 3 months and 6 months after initiation of the biological drug; side effects; psoriatic arthritis; concomitant methotrexate treatment; drug name; and

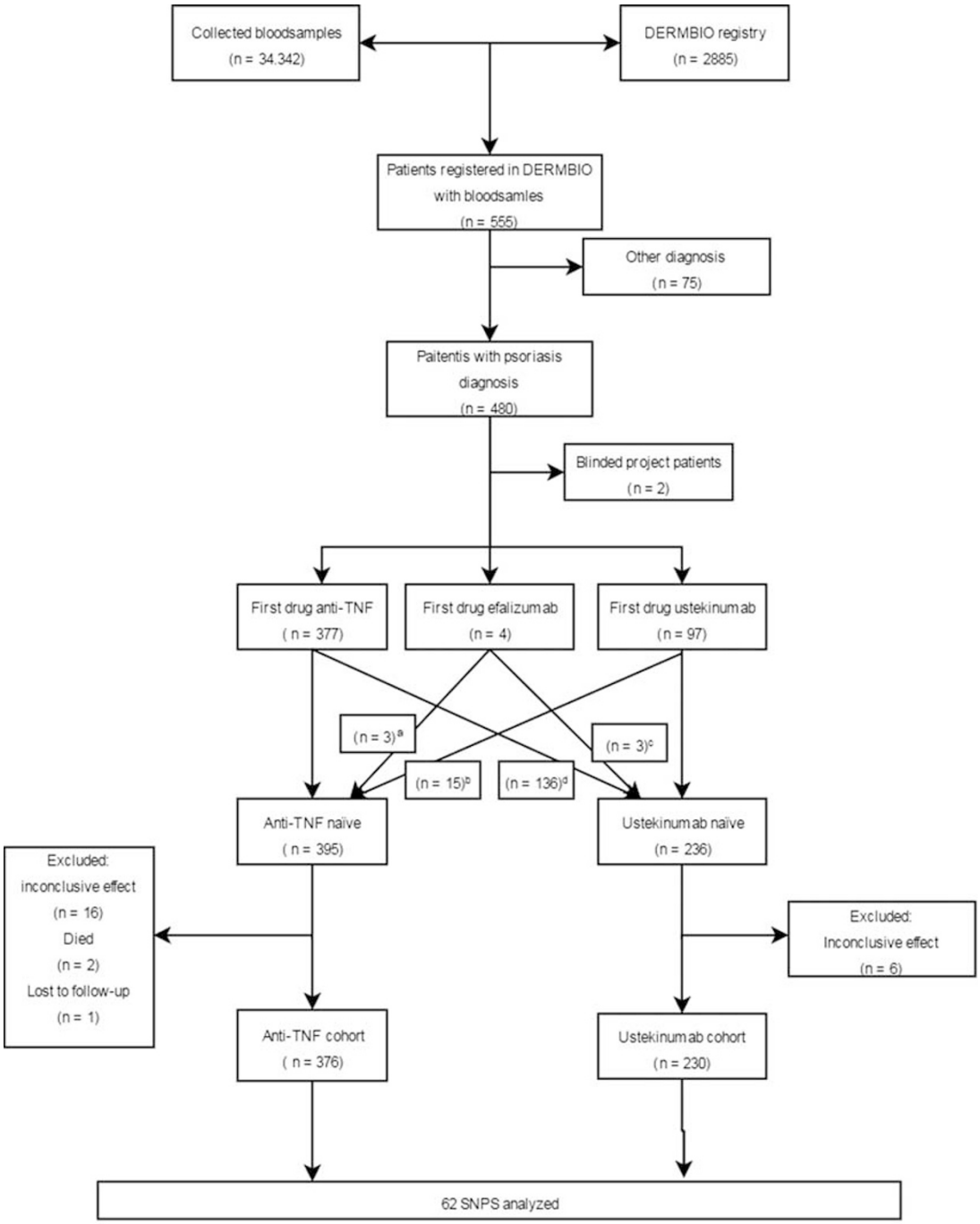

Figure 1. Selection of patients included in the study.

treatment number. The baseline was defined as day of start of treatment (±14 days), the 3-month visit as 90 days (±45 days) and the 6-month visit as 180 days (±45 days). If more visits were recorded within the periods, the ones closest to the baseline and 3- and 6-month marks were chosen.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local regional ethics committees (M-20100153 and S-20120113) and the Danish Data Protection Agency (J. 2010-41-4719 and 2008-58-035). The regional ethics committees gave exemption from obtaining informed consent because information had no health-related impact on subjects.

Response criteria

We chose drug survival (the period of time that a patient receives biological treatment) to be our *primary response criterion*. Patients treated for 225 days (equivalent to 6 months+45 days) or more were categorized as responders. Patients who stopped treatment before 225 days were categorized as either primary nonresponders or secondary nonresponders. Patients who initially had a response (a relative reduction in PASI of at least 75%—or information from the doctor/health personnel in the patient records that a beneficial effect had been achieved), but where the response diminished over time or treatment was stopped for other reasons (for example, side effects), were categorized as secondary nonresponders, and those who never responded to treatment were categorized as primary nonresponders. Patients who stopped treatment before 225 days for other reasons than lack of effect, and where no information of response was available, were excluded from further analysis.

As our *secondary response criteria*, we evaluated response based on relative reduction in PASI score after 3 months. Patients who achieved reduction of $<50\%$ were categorized as being nonresponders, those with a reduction of between 50 and 75% were categorized as being intermediate responders and those with a reduction of $\geqslant 75\%$ were categorized as being good responders. Patients with missing baseline PASI but a PASI score of 0 after 3 months (equivalent to a PASI reduction of 100%) were categorized as good responders.

Genotyping

We selected genes involved in nuclear factor-κB, TNF-α and pattern recognition pathways as described by Bank *et al.*[6] and Sode *et al.*[8] Briefly, candidates were found by searching for '*polymorphism AND Gene name AND (reporter gene OR luciferase OR ELISA OR RT-PCR OR flow cytometry OR EMSA)*' and the single-nucleotide polymorphism (SNP) candidates were chosen based on the reported functionality, association with autoimmune diseases or association with response.[6–8] A list of all SNPs studied is presented in Supplementary Table 1a. DNA extraction (Maxwell 16 LEV Blood DNA Kit; Promega, Madison, WI, USA) was performed as described by Bank *et al.*[13]

The polymorphisms were genotyped with PCR-based KASP genotyping assays by LGC Genomics (LGC Genomics, Hoddesdon, UK; http://www.lgcgenomics.com/). *FGF2* (rs308379) and *TLR10* (rs11096957) were only genotyped in 376 patients; the rest of the SNPs were genotyped in all patients.

Linking disequilibrium was calculated using SNAP (http://archive.broadinstitute.org/mpg/snap/) using as reference the Central Europeans in the 1000 Genomes.[14]

As a quality control, all SNPs were replicated in 94 randomly selected samples, yielding $>99\%$ identical genotypes. The average call rate for all SNPs was $>97\%$. None of the patients had the minor allele of *IL-12RB2* (rs11810249). *IL-18* (rs187238) and *IL-18* (rs360719) were in complete linking disequilibrium ($r^2 = 1.00$), and rs360719 was therefore excluded from further analysis.

Power analysis

At the 5% significance level and a minor allele frequency of 0.05, 0.25 and 0.45, we had $>80\%$ power of detecting a dominant effect with an odds ratio (OR) of 2.0, 1.8 and 2.2, respectively, for the anti-TNF cohort and an OR of 2.3, 2.2 and 3.3, respectively, for the ustekinumab cohort. The Genetic Power Calculator[15] was used for power calculations, setting 'prevalence' to 0.33, d' to 1, type 1 error rate to 0.05 and number of cases and control/case ratio was based on data described in Supplementary Table 2a.

Statistical analysis

Under a dominant model logistic regression analysis was used to compare genotypes in responders against those in primary nonresponders (R vs PN), and genotypes in responders and secondary nonresponders combined against those in primary nonresponders (R+S vs PN), as genetics may not have main effects on secondary nonresponse. For our *second outcome*, we compared good responders against nonresponders (G vs N) and good and intermediate responders against nonresponders (G+I vs N) under a dominant model. Crude ORs and ORs adjusted for gender, age, psoriatic arthritis and previous biological treatment are reported (Supplementary Tables 3). Genotype distributions for all patients and for each outcome tested are presented in Supplementary Table 1b. We corrected for multiple testing by controlling the overall false discovery rate (FDR) at 20%,[16] with computation of FDR-adjusted *P*-values (referred to as *q*-values) based on all *P*-values presented in Supplementary Tables 3. The *q*-value describes the expected proportion of false positives among all associations at least as extreme as the observed association. The χ^2 test was used to compare differences in categorical outcomes of clinical and demographic characteristics, and for continuous outcomes a one-way analysis of variance was used. Statistical analyses were performed using Stata version 14 (StataCorp LP, College Station, TX, USA). Computation of *q*-values was performed using the inbuilt p-adjust function in R (Vienna, Austria) using the Benjamini–Hochberg method.

RESULTS

Study population

A total of 480 patients with blood clot samples available were registered in DERMBIO as having psoriasis. Two patients were excluded because of participation in blinded clinical trials, leaving 478 patients with 631 treatment series. These treatment series involved 395 patients treated with anti-TNF and 236 patients treated with ustekinumab. Nineteen of the patients treated with anti-TNF were excluded from analysis because of inconclusive responses, resulting in a cohort of 376 patients treated with anti-TNF. Six of the patients treated with ustekinumab were excluded from analysis because of inconclusive responses, resulting in a cohort of 230 patients treated with ustekinumab (Figure 1). The clinical and demographic characteristics of all patients are given in Table 1, including stratification for treatment. Characteristics of all patients registered in DERMBIO are presented in Supplementary Table 2c.

Patients treated with anti-TNF

Looking at *primary outcome* of the 376 patients treated with anti-TNF, 294 (78%) were responders, 30 (8%) were secondary nonresponders and 52 (14%) were primary nonresponders (Supplementary Table 2a). Statistically fewer females than males responded when treated with anti-TNF ($P < 0.001$). Regarding *secondary outcome*, among patients treated with anti-TNF, 250 had PASI scores available at baseline and after 3 months. Of these, 163 (65%) were good responders, 41 (16%) were intermediate responders and 46 (18%) were nonresponders (Supplementary Table 2b). Previous treatment with ustekinumab was associated with nonresponse ($P = 0.02$).

Polymorphisms associated with anti-TNF treatment

Associations of each SNP and response to anti-TNF therapy were evaluated using drug survival as outcome, comparing genotypes of responders with those of primary nonresponders under a dominant model. Four of the analyzed SNPs showed nominal statistical significance ($P < 0.05$) for both crude and adjusted ORs (Supplementary Table 3). After controlling for the FDR, only *TLR9* (rs352139) (OR: 2.42, $P = 0.0044$, $q = 0.19$) remained significant (Table 2). Associations of each SNP and response to anti-TNF therapy were then evaluated using PASI reduction as outcome, comparing genotypes of good responders with those of nonresponders under a dominant model. Six of the analyzed SNPs

Table 1. Clinical and demographic characters for all the patients included, the anti-TNF cohort and the ustekinumab cohort

	All patients	*Anti-TNF cohort*	*Ustekinumab cohort*
Patients, no.	478	376	230
Female, no. (%)	193 (40)	145 (39)	107 (46)
Age, years			
Age at baseline, mean (s.d.)	44 (14)	43 (14)	44 (14)
Disease duration, years			
Duration at baseline, mean (s.d.)	20 (13)	20 (13)	21 (13)
	A = 295 (62%)	A = 248 (66%)	A = 133 (58%)
BMI at baseline, mean (s.d.)	28 (6)	28 (6)	29 (6)
	A = 177 (37%)	A = 146 (39%)	A = 102 (44%)
Psoriatic arthritis, no. (%)	148 (31)	126 (34)	50 (22)
Concomitant methotrexate, no. (%)	67 (16)	55 (16)	28 (13)
	A = 432 (90%)	A = 340 (90%)	A = 220 (96%)
Biological treatment, no. (%)			
Adalimumab	216 (45.19)	218 (57.98)	—
Etanercept	106 (22.18)	103 (27.39)	—
Infliximab	55 (11.51)	55 (14.61)	—
Ustekinumab	97 (20.29)	—	230 (100)
Efalizumab	4 (0.84)	—	—
Biological naive	—	358 (95)	95 (41)
PASI scores, mean (s.d.)			
PASI at baseline	13.2 (7.8)	13.5 (7.8)	9.9 (7.2)
	A = 365 (76%)	A = 297 (79%)	A = 185 (80%)
PASI at 3 months	3.0 (4.0)	3.2 (4.2)	2.9 (3.6)
	A = 329 (69%)	A = 269 (72%)	A = 165 (72%)
PASI at 6 months	2.3 (3.5)	2.2 (3.1)	2.4 (3.4)
	A = 291 (61%)	A = 233 (62%)	A = 146 (63%)

Abbreviations: A, available observations; BMI, body mass index; PASI, Psoriasis Area and Severity Index; TNF, tumor necrosis factor.

showed nominal statistical significance ($P < 0.05$) for both crude and adjusted ORs (Supplementary Table 4). After controlling for the FDR, 5 of the SNPs remained significant, whereof the variant alleles of *IL1B* (rs1143623) (OR: 0.35, $P = 0.0041$, $q = 0.19$), *IL1B* (rs1143627; $r^2 = 0.83$) (OR: 0.28, $P = 0.0016$, $q = 0.19$), *LY96* (rs11465996) (OR: 0.33, $P = 0.0044$, $q = 0.19$), *TLR2* (rs11938228) (OR: 0.30, $P = 0.0019$, $q = 0.19$) and *TLR2* (rs4696480; $r^2 = 0.48$) (OR: 0.22, $P = 0.0032$, $q = 0.19$) were all associated with nonresponse to treatment (Table 2).

Patients treated with ustekinumab

Looking at *primary outcome* of the 230 patients treated with ustekinumab, 201 (87%) were responders, 2 (1%) were secondary nonresponders and 27 (12%) were primary nonresponders (Supplementary Table 2a). Previous treatment with anti-TNF was associated with nonresponse ($P = 0.02$). Regarding *secondary outcome*, of the patients treated with ustekinumab, 143 had PASI scores available at baseline and after 3 months. Of these, 80 (56%) were good responders, 29 (20%) were intermediate responders and 34 (24%) were nonresponders (Supplementary Table 2b) Previous treatment with anti-TNF was associated with nonresponse ($P = 0.005$).

Polymorphisms associated with ustekinumab treatment

We then evaluated associations between SNPs and response to ustekinumab therapy using drug survival as outcome, comparing genotypes of responders with those of primary nonresponders. Five of the analyzed SNPs showed nominal statistical significance ($P < 0.05$) for both crude and adjusted ORs (Supplementary Table 5), none of which remained significant after controlling for FDR. Next, associations of each SNP and response to ustekinumab therapy were evaluated using PASI reduction as outcome, comparing genotypes of good responders with those of nonresponders under a dominant model. Six of the analyzed SNPs showed nominal statistical significance ($P < 0.05$) for both crude and adjusted ORs (Supplementary Table 6). Four associations remained significant after controlling for FDR, the variant alleles of *TIRAP* (rs8177374) (OR: 9.42, $P = 0.0051$, $q = 0.19$) and *TLR5* (rs5744174) (OR: 5.26, $P = 0.0012$, $q = 0.19$) were associated with beneficial response and the variant alleles of *IL1B* (rs1143623) (OR: 0.25, $P = 0.0049$) and *IL1B* (rs1143627; $r^2 = 0.83$) (OR: 0.24, $P = 0.0042$, $q = 0.19$) were associated with nonresponse (Table 2).

Haplotype analysis for both treatments

Polymorphisms in the *IL1B* gene were associated with response to both treatments when we looked at the *secondary outcome*. To assess whether any haplotypes could explain these associations, we performed haplotype analysis with PASI reduction as the response criterion (Supplementary Tables 7 and 8). Four haplotypes described all of the observed genotypes in both groups, excluding five patients who were treated with anti-TNF and four patients who were treated with ustekinumab because of missing genotype data. With haplotype combination 11 (rs4848306AA, rs1143623GG and rs1143627TT) as reference, the haplotype combinations 22 (rs4848306GG, rs1143623CC and rs1143627CC) (OR: 0.22, 95% confidence interval: 0.05–0.95, $P = 0.043$) and 12 (rs4848306AG, rs1143623GC and rs1143627TC) (OR: 0.28, 95% confidence interval: 0.09–0.84, $P = 0.023$) were associated with nonresponse to anti-TNF (Supplementary Table 7). Haplotype combination 12 (OR: 0.12, 95% confidence interval: 0.03–0.54, $P = 0.006$) was associated with nonresponse to ustekinumab (Supplementary Table 8).

Table 2. Crude and adjusted OR for carriers of the variant allele compared with homozygous carriers of the wild-type allele for SNPs significant controlling the FDR at 20% when treating psoriasis with anti-TNF or ustekinumab

Gene (W/Vt)	*Rs number*	*OR (95% CI),* q-*value/crude*	*OR (95% CI),* q-*value/adjusted*[a]	*Effect of variant allele*
Response to anti-TNF treatment based on drug survival (R vs PN)				
TLR9				
G/A	rs352139	2.42 (1.32–4.44), 0.19	2.17 (1.15–4.09), 0.33	Increased expression of TLR9[52 b]
Response to anti-TNF treatment based on PASI reduction after 3 months (G vs *N)*				
IL1B				
G/C	rs1143623	0.42 (0.21–0.83), 0.30	0.35 (0.17–0.71), 0.19	Decreased IL-1β[19 c] but is only found in haplotypes with increased IL-1β transcription[21 b]
T/C	rs1143627	0.36 (0.17–0.74), 0.19	0.28 (0.13–0.62), 0.19	Decreased expression[19,20 c,d] but is only seen in haplotypes with increased IL-1β transcription[21 b]
LY96				
C/G	rs11465996	0.38 (0.19–0.77), 0.23	0.33 (0.15–0.71), 0.19	Increased MD-2 and TNF-α level[17 e, c]
TLR2				
C/A	rs11938228	0.36 (0.18–0.74), 0.19	0.30 (0.14–0.64), 0.19	Unknown
A/T	rs4696480	0.31 (0.12–0.78), 0.19	0.22 (0.08–0.59), 0.19	Unknown
Response to ustekinumab treatment based on PASI reduction after 3 months (G vs *N)*				
IL1B				
G/C	rs1143623	0.21 (0.09–0.52), 0.19	0.25 (0.09–0.66), 0.19	Decreased IL-1β[19 c] but is only found in haplotypes with increased IL-1β transcription[21 b]
T/C	rs1143627	0.24 (0.09–0.63), 0.19	0.25 (0.09–0.73), 0.30	Decreased expression[19,20 c, f] but is only seen in haplotypes with increased IL-1β transcription[21 b]
TIRAP				
C/T	rs8177374	8.62 (1.92–38.64), 0.19	9.42 (1.96–45.30), 0.19	Increased TNF-α, IFN-γ and IL-6[27 c]
TLR5				
T/C	rs5744174	4.43 (1.86–10.56), 0.19	5.26 (1.93–14.38), 0.19	Decreased mRNA levels of IL-6 and IL-1β[23 d], increased production of CCL20[24 c] and IFN-γ level[25 c]

Abbreviations: CI, confidence interval; FDR, false discovery rate; G vs N, good responders (⩾PASI75) vs nonresponders (<PASI50); IFN-γ, interferon-γ; IL, interleukin; OR, odds ratio (>1.00 associated with beneficial response); PASI, Psoriasis Area and Severity Index; R vs PN, responders (⩾225 days of treatment) vs primary nonresponders (<225 days of treatment and no effect of treatment); SNP, single-nucleotide polymorphism; TLR, Toll-like receptor; TNF, tumor necrosis factor; Vt, variant allele; W, wild-type allele. [a]Adjusted for age, gender, psoriatic arthritis and previous treatments. [b]Function examined by luciferase reporter assay. [c]Function examined by enzyme-linked immunosorbent assay. [d]Function examined by electrophoretic mobility shift assay. [e]Function examined by flow cytometry. [f]Function examined by real-time PCR.

DISCUSSION

Here, 62 SNPs in 44 genes were successfully genotyped. With a FDR of <0.20 we identified 6 SNPs that were associated with responses to anti-TNF drug therapy, 4 that were associated with responses to ustekinumab, and 2 of which were associated with responses to both treatments. To our knowledge, none of the SNPs identified to be associated with response to psoriasis treatment have been reported previously.

The variant allele of *LY96* (rs11465996), related to a high level of MD-2 (ref. 17) (required for TLR2 and TLR4 to respond to lipopolysaccharide), was associated with nonresponse to anti-TNF therapy. Two SNPs in *TLR2* (rs11938228 and rs4696480) with unknown function were associated with nonresponse, and this might indicate that TLR2 activity is associated with anti-TNF treatment, although the unknown function of the two SNPs makes the results difficult to interpret in a biological context. Similar associations for *TLR2* (rs11938228, rs4696480) have been found when treating inflammatory bowel disease with anti-TNF, and for *LY96* (rs11465996) when treating ulcerative colitis with anti-TNF,[6] supporting the notion of a possible association. Furthermore, a decrease in TLR2 levels has been found to be correlated with a decrease in PASI when treating psoriasis with anti-TNF agents[18] that could support the current findings.

In patients treated with anti-TNF, the variant alleles of *IL1B* (rs1143623 and rs1143627) were associated with nonresponse. In single-polymorphism context the variant alleles of these two SNPs are associated with reduced IL-1β transcription,[19,20] but in the haplotype context, which is the dominating haplotype in Caucasians, the variant alleles of these two SNPs led to increased transcription of IL-1β,[21] indicating that a genetically determined high level of IL-1β is associated with nonresponse to anti-TNF treatment. In agreement with our current findings, an elevated IL-1β level has been associated with an unfavorable response to infliximab in inflammatory bowel disease.[22] In our study, patients with haplotypes associated with increased transcription of IL-1β were less likely to respond to anti-TNF treatment,[21] further supporting the findings. In patients treated with ustekinumab, the same associations for *IL1B* (rs1143623 and rs1143627) were seen, and the same haplotype conveying increased IL-1β transcription was associated with nonresponse. The variant allele of *TLR5* (rs5744174), which is related to decreased IL-1β and IL-6 mRNA,[23] and increased levels of CCL20 (ref. 24) and IFN-γ[25] were associated with beneficial response to ustekinumab, again indicating that an increased IL-1β level may be unfavorable when treating psoriasis with ustekinumab. However, the variant allele of *TLR2* (rs3804099), which is related to high levels of TNF-α, IL-1β and IL-6 and a reduced MYD88 level,[26] was nominally associated with a beneficial response, and this might indicate that not only the level of IL-1β but also the relative abundance of cytokines is crucial for response to ustekinumab treatment.

For patients treated with ustekinumab, variants related to an increased level of IFN-γ (*TLR5* (rs5744174)[25] and *TIRAP* (rs8177374)[27]) appears to be associated with a beneficial response. Moreover, the variant allele of *IFNG* (rs2430561)—conveying high levels of IFN-γ[28,29] —was found to be nominally associated with a beneficial response to ustekinumab. Furthermore, when we adjusted for gender, age, psoriatic arthritis and previous treatments, the variant allele of *TBX21* (rs17250932), which is related to decreased IFN-γ level,[30] was nominally associated with nonresponse. IFN-γ is highly upregulated in psoriasis and is one of the

key cytokines.[2] A decrease in IFN-γ levels has been shown to be correlated with a decrease in PASI scores.[31] By blocking IL-12p40, ustekinumab blocks the IL-12-dependent Th1 production of IFN-γ that could explain why a genetically determined elevated level of IFN-γ is associated with a beneficial response. However, we cannot exclude the possibility that the associations observed are because of factors other than the increased IFN-γ. Moreover, our results are supported by a Dutch pilot study[32] in which patients who responded to ustekinumab had a strong IFN signature compared with that of nonresponders.

In order to increase the statistical power, we analyzed all anti-TNF treatments as one, a commonly used strategy.[33–43] For all significant associations, except *TLR9* (rs352139) and *LY96* (rs11465996), similar tendencies were observed for ORs for each of the anti-TNF agents (data available on request). To our knowledge, this is one of the largest cohorts for evaluation of response in patients treated with ustekinumab[44,45] and the second largest for evaluation of anti-TNF; only a Canadian study (also involving treatment primarily for psoriatic arthritis) had more patients.[46] In the present study, some of the patients were included in both the ustekinumab cohort and the anti-TNF cohort; however, as the treatments target different pathways, including previously treated patients is a commonly used approach.[47–50] Efficacy of treatments was evaluated based on both drug survival of 6 months and relative PASI reduction after 3 months. We cannot exclude that categorization of response for some of the patients may be influenced by other factors, for example, comedication, patients' preferences or dose changes, although this is highly unlikely as these factors primarily affect the overall long-term drug survival and not the short-term response. Even though drug survival was our primary outcome, the majority of the associations were observed evaluating treatment based on PASI that could indicate outcomes based on PASI scores to be more suitable in future studies.

With increasing number of test performed, the risk of false positive associations similarly increases. To take this into account, we corrected for multiple testing by controlling the false discovery rate at 0.20. By doing so we found that eight of the SNPs evaluated were associated with response to one or both treatments and the majority were functionally and biologically plausible according to current knowledge and previous findings. Given the number of significant associations and the selected FDR of 0.20, approximately two of the reported associations would be expected to be false positives. However, by choosing functional polymorphisms possibly involved in the pathogenesis and treatment of psoriasis, we increased the *a priori* likelihood of true associations. The SNPs were evaluated for genotyping errors by assessing Hardy–Weinberg equilibrium. This was performed in a control group of healthy Danish blood donors[51] as Hardy–Weinberg equilibrium cannot be assessed in the current highly selected study group of patients with psoriasis selected for treatment with biological therapy. The control group was genotyped by the same method at the same laboratory. All SNPs were in Hardy–Weinberg equilibrium. The overall characteristics of the current group resample that of the entire DERMBIO database, apart from a slightly higher proportion of female patients in the current study.

In conclusion, we have shown that genetic variants in genes that regulate cytokines involved in psoriasis are associated with response when psoriasis is treated with either anti-TNF or ustekinumab. Genetic variants related to elevated levels of IL-1β appear to be unfavorable when treating psoriasis with either anti-TNF or ustekinumab, whereas patients with genetic variants related to a high level of IFN-γ appear to be more likely to respond when being treating with ustekinumab. As none of our results have been associated with response to biological treatment of psoriasis previously, they should be validated in independent cohorts.

CONFLICT OF INTEREST

Dr Skov has received consultancy and/or speaker honoraria from Abbvie, Pfizer, Janssen-Cilag, Merck Sharp & Dohme (MSD) and LEO Pharma and is a member of the advisory boards of Abbvie, Pfizer, Janssen-Cilag, MSD, Eli Lilly, Celgene and Novartis. Dr Iversen has been a paid speaker for MSD, Pfizer, AbbVie, Almirall, Janssen-Cilag, Eli Lilly, Novartis and LEO Pharma. He has been consulting or serving on expert/advisory boards with Pfizer, AbbVie, Almirall, BMS, Janssen-Cilag, Novartis, Eli Lilly, LEO Pharma and MSD. He has served as investigator for MSD, Pfizer, AbbVie, Janssen-Cilag, Eli Lilly, Novartis, Amgen and LEO Pharma and has received research and educational grant from Pfizer, AbbVie, Novartis, MSD and LEO Pharma. Dr Gniadecki has received consultancy and speaker honoraria from Abbvie, Janssen-Cilag and Novartis and is a member of the advisory boards of Abbvie, Amgen, Janssen-Cilag, Eli Lilly, Celgen, Novartis and Therakos. Dr Dam has received compensation as a speaker and member of an advisory board for Janssen-Cilag and Abbvie. Dr Andersen has received compensation as a consultant and member of an advisory board for MSD and Janssen-Cilag.

ACKNOWLEDGMENTS

The work was funded by Psoriasisforeningen, Robert Wehnerts og Kirsten Wehnerts Fonden, Knud og Edith Eriksens Mindefond, Gigtforeningen (A2037, A3570) and the Novo Nordisk Foundation (NNF15OC0017622).

REFERENCES

1 Parisi R, Symmons DPM, Griffiths CEM, Ashcroft DM. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. *J Invest Dermatol* 2013; **133**: 377–385.
2 Baliwag J, Barnes DH, Johnston A. Cytokines in psoriasis. *Cytokine* 2015; **73**: 342–350.
3 Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med* 2009; **361**: 496–509.
4 Nast A, Jacobs A, Rosumeck S, Werner RN. Efficacy and safety of systemic long-term treatments for moderate-to-severe psoriasis: a systematic review and meta-analysis. *J Invest Dermatol* 2015; **135**: 2641–2648.
5 Prieto-Pérez R, Cabaleiro T, Daudén E, Ochoa D, Roman M, Abad-Santos F. Genetics of psoriasis and pharmacogenetics of biological drugs. *Autoimmune Dis* 2013; **2013**: 613086.
6 Bank S, Andersen PS, Burisch J, Pedersen N, Roug S, Galsgaard J *et al.* Associations between functional polymorphisms in the NF[kappa]B signaling pathway and response to anti-TNF treatment in Danish patients with inflammatory bowel disease. *Pharmacogenomics J* 2014; **14**: 526–534.
7 Bank S, Skytt Andersen P, Burisch J, Pedersen N, Roug S, Galsgaard 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**: e98815.
8 Sode J, Vogel U, Bank S, Andersen PS, Thomsen MK, Hetland ML *et al.* Anti-TNF treatment response in rheumatoid arthritis patients is associated with genetic variation in the NLRP3-inflammasome. *PLoS ONE* 2014; **9**: e100361.
9 Sode J, Vogel U, Bank S, Andersen PS, Hetland ML, Locht H *et al.* Confirmation of an IRAK3 polymorphism as a genetic marker predicting response to anti-TNF treatment in rheumatoid arthritis. *Pharmacogenomics J* 2016; doi: 10.1038/tpj.2016.66.
10 Bank S, Andersen PS, Burisch J, Pedersen N, Roug S, Galsgaard 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. *Pharmacogenomics J* 2016; doi: 10.1038/tpj.2016.84 (in press).
11 Gniadecki R, Bang B, Bryld L, Iversen L, Lasthein S, Skov L. Comparison of long-term drug survival and safety of biologic agents in patients with psoriasis vulgaris. *Br J Dermatol* 2015; **172**: 244–252.
12 Fredriksson T, Pettersson U. Severe psoriasis--oral therapy with a new retinoid. *Dermatologica* 1978; **157**: 238–244.
13 Bank S, Nexø 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 Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, De Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 2008; **24**: 2938–2939.
15 Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003; **19**: 149–150.
16 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B (Methodol)* 1995; **57**: 289–300.

17 Gu W, Shan Y-a, Zhou J, Jiang D-p, Zhang L, Du D-y *et al.* Functional significance of gene polymorphisms in the promoter of myeloid differentiation-2. *Ann Surg* 2007; **246**: 151–158.
18 Vageli DP, Exarchou A, Zafiriou E, Doukas PG, Doukas S, Roussaki-Schulze A. Effect of TNF-α inhibitors on transcriptional levels of pro-inflammatory interleukin-33 and Toll-like receptors-2 and -9 in psoriatic plaques. *Exp Ther Med* 2015; **10**: 1573–1577.
19 Wen A-Q, Gu W, Wang J, Feng K, Qin L, Ying C *et al.* Clincal relevance of IL-1beta promoter polymorphisms (−1470, − 511, and − 31) in patients with major trauma. *Shock* 2010; **33**: 576–582.
20 Lind H, Haugen A, Zienolddiny S. Differential binding of proteins to the IL1B − 31T/C polymorphism in lung epithelial cells. *Cytokine* 2007; **38**: 43–48.
21 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.
22 Lacruz-Guzmán D, Torres-Moreno D, Pedrero F, Romero-Cara P, García-Tercero I, Trujillo-Santos J *et al.* Influence of polymorphisms and TNF and IL1β serum concentration on the infliximab response in Crohn's disease and ulcerative colitis. *Eur J Clin Pharmacol* 2013; **69**: 431–438.
23 Klimosch SN, Försti A, Eckert J, Knežević J, Bevier M, von Schönfels W *et al.* Functional TLR5 genetic variants affect human colorectal cancer survival. *Cancer Res* 2013; **73**: 7232.
24 Sheridan J, Mack DR, Amre DK, Israel DM, Cherkasov A, Li H *et al.* A non-synonymous coding variant (L616F) in the TLR5 gene is potentially associated with Crohn's disease and influences responses to bacterial flagellin. *PLoS ONE* 2013; **8**: e61326.
25 Dhiman N, Ovsyannikova IG, Vierkant RA, Ryan JE, Pankratz VS, Jacobson RM *et al.* Associations between SNPs in Toll-like receptors and related intracellular signaling molecules and immune responses to measles vaccine: preliminary results. *Vaccine* 2008; **26**: 1731–1736.
26 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.
27 Ferwerda B, Alonso S, Banahan K, McCall MBB, Giamarellos-Bourboulis EJ, Ramakers BP *et al.* Functional and genetic evidence that the Mal/TIRAP allele variant 180L has been selected by providing protection against septic shock. *Proc Natl Acad Sci USA* 2009; **106**: 10272–10277.
28 Schena FP, Cerullo G, Torres DD, Scolari F, Foramitti M, Amoroso A *et al.* Role of interferon-[gamma] gene polymorphisms in susceptibility to IgA nephropathy: a family-based association study. *Eur J Hum Genet* 2006; **14**: 488–496.
29 Pravica V, Perrey C, Stevens A, Lee J-H, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-γ gene:: Absolute correlation with a polymorphic CA microsatellite marker of high IFN-γ production. *Hum Immunol* 2000; **61**: 863–866.
30 Li J, Li J, You Y, Chen S. The role of upstream stimulatory factor 1 in the transcriptional regulation of the human TBX21 promoter mediated by the T-1514C polymorphism associated with systemic lupus erythematosus. *Immunogenetics* 2012; **64**: 361–370.
31 Abdallah MA, Abdel-Hamid MF, Kotb AM, Mabrouk EA. Serum interferon-gamma is a psoriasis severity and prognostic marker. *Cutis* 2009; **84**: 163–168.
32 Onderdijk AJ, Ijpma AS, Menting SP, Baerveldt EM, Prens EP. Potential serum biomarkers of treatment response to ustekinumab in patients with psoriasis: a pilot study. *Br J Dermatol* 2015; **173**: 1536–1539.
33 Di Renzo L, Bianchi A, Saraceno R, Calabrese V, Cornelius C, Iacopino L *et al.* -174G/C IL-6 gene promoter polymorphism predicts therapeutic response to TNF-alpha blockers. *Pharmacogenet Genomics* 2012; **22**: 134–142.
34 Ryan C, Kelleher J, Fagan MF, Rogers S, Collins P, Barker JN *et al.* Genetic markers of treatment response to tumour necrosis factor-alpha inhibitors in the treatment of psoriasis. *Clin Exp Dermatol* 2014; **39**: 519–524.
35 Julia M, Guilabert A, Lozano F, Suarez-Casasus B, Moreno N, Carrascosa JM *et al.* The role of Fcgamma receptor polymorphisms in the response to anti-tumor necrosis factor therapy in psoriasis A pharmacogenetic study. *JAMA Dermatol* 2013; **149**: 1033–1039.
36 Gallo E, Cabaleiro T, Román M, Solano-López G, Abad-Santos F, García-Díez A *et al.* The relationship between tumour necrosis factor (TNF)-α promoter and IL12B/IL-23R genes polymorphisms and the efficacy of anti-TNF-α therapy in psoriasis: a case–control study. *Br J Dermatol* 2013; **169**: 819–829.
37 Nishikawa R, Nagai H, Bito T, Ikeda T, Horikawa T, Adachi A *et al.* Genetic prediction of the effectiveness of biologics for psoriasis treatment. *J Dermatol* 2016; **43**: 1273–1277.
38 Coto-Segura P, Batalla A, Gonzalez-Fernandez D, Gomez J, Santos-Juanes J, Queiro R *et al.* CDKAL1 gene variants affect the anti-TNF response among Psoriasis patients. *Int Immunopharmacol* 2015; **29**: 947–949.
39 Gonzalez-Lara L, Batalla A, Coto E, Gomez J, Eiris N, Santos-Juanes J *et al.* The TNFRSF1B rs1061622 polymorphism (p.M196R) is associated with biological drug outcome in psoriasis patients. *Arch Dermatol Res* 2015; **307**: 405–412.
40 Prieto-Perez R, Solano-Lopez G, Cabaleiro T, Roman M, Ochoa D, Talegon M *et al.* New polymorphisms associated with response to anti-TNF drugs in patients with moderate-to-severe plaque psoriasis. *Pharmacogenomics J* 2016; doi: 10.1038/tpj.2016.64.
41 Batalla A, Coto E, Gomez J, Eiris N, Gonzalez-Fernandez D, Gomez-De Castro C *et al.* IL17RA gene variants and anti-TNF response among psoriasis patients. *Pharmacogenomics J* 2016; doi: 10.1038/tpj.2016.70.
42 Julia A, Ferrandiz C, Dauden E, Fonseca E, Fernandez-Lopez E, Sanchez-Carazo JL *et al.* Association of the PDE3A-SLCO1C1 locus with the response to anti-TNF agents in psoriasis. *Pharmacogenomics J* 2015; **15**: 322–325.
43 Masouri S, Stefanaki I, Ntritsos G, Kypreou KP, Drakaki E, Evangelou E *et al.* A Pharmacogenetic study of psoriasis risk variants in a Greek population and prediction of responses to anti-TNF-alpha and anti-IL-12/23 agents. *Mol Diagn Ther* 2016; **20**: 221–225.
44 Li K, Huang CC, Randazzo B, Li S, Szapary P, Curran M *et al.* HLA-C*06:02 allele and response to IL-12/23 inhibition: results from the Ustekinumab Phase 3 Psoriasis Program. *J Invest Dermatol* 2016; **136**: 2364–2371.
45 Talamonti M, Galluzzo M, van den Reek JM, de Jong EM, Lambert JL, Malagoli P *et al.* Role of HLA- C*06 in clinical response to ustekinumab: evidence from real-life in a large cohort of European patients. *Br J Dermatol* 2017; doi: 10.1111/bjd.15387.
46 Tejasvi T, Stuart PE, Chandran V, Voorhees JJ, Gladman DD, Rahman P *et al.* TNFAIP3 gene polymorphisms are associated with response to TNF blockade in psoriasis. *J Invest Dermatol* 2012; **132**: 593–600.
47 van den Reek JM, Coenen MJ, van de L'Isle Arias M, Zweegers J, Rodijk-Olthuis D, Schalkwijk J *et al.* Polymorphisms in CD84, IL12B and TNFAIP3 are associated with response to biologics in patients with psoriasis. *Br J Dermatol* 2016; **176**: 1288–1296.
48 Galluzzo M, Boca AN, Botti E, Potenza C, Malara G, Malagoli P *et al.* IL12B (p40) gene polymorphisms contribute to ustekinumab response prediction in psoriasis. *Dermatology* 2016; **232**: 230–236.
49 Chiu HY, Wang TS, Chan CC, Cheng YP, Lin SJ, Tsai TF. Human leucocyte antigen-Cw6 as a predictor for clinical response to ustekinumab, an interleukin-12/23 blocker, in Chinese patients with psoriasis: a retrospective analysis. *Br J Dermatol* 2014; **171**: 1181–1188.
50 Talamonti M, Botti E, Galluzzo M, Teoli M, Spallone G, Bavetta M *et al.* Pharmacogenetics of psoriasis: HLA-Cw6 but not LCE3B/3C deletion nor TNFAIP3 polymorphism predisposes to clinical response to interleukin 12/23 blocker ustekinumab. *Br J Dermatol* 2013; **169**: 458–463.
51 Bank S, Andersen PS, Burisch J, Pedersen N, Roug S, Galsgaard 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**: e0145302.
52 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.

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