

Rheumatoid Arthritis Risk Allele *PTPRC* Is Also Associated With Response to Anti-Tumor Necrosis Factor α Therapy

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Objective. Anti-tumor necrosis factor α (anti-TNF) therapy is a mainstay of treatment in rheumatoid arthritis (RA). The aim of the present study was to test established RA genetic risk factors to determine whether the same alleles also influence the response to anti-TNF therapy.

Methods. A total of 1,283 RA patients receiving

etanercept, infliximab, or adalimumab therapy were studied from among an international collaborative consortium of 9 different RA cohorts. The primary end point compared RA patients with a good treatment response according to the European League Against Rheumatism (EULAR) response criteria (n = 505) with

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RA patients considered to be nonresponders ($n = 316$). The secondary end point was the change from baseline in the level of disease activity according to the Disease Activity Score in 28 joints (Δ DAS28). Clinical factors such as age, sex, and concomitant medications were tested as possible correlates of treatment response. Thirty-one single-nucleotide polymorphisms (SNPs) associated with the risk of RA were genotyped and tested for any association with treatment response, using univariate and multivariate logistic regression models.

Results. Of the 31 RA-associated risk alleles, a SNP at the *PTPRC* (also known as *CD45*) gene locus (rs10919563) was associated with the primary end point, a EULAR good response versus no response (odds ratio [OR] 0.55, $P = 0.0001$ in the multivariate model). Similar results were obtained using the secondary end

point, the Δ DAS28 ($P = 0.0002$). There was suggestive evidence of a stronger association in autoantibody-positive patients with RA (OR 0.55, 95% confidence interval [95% CI] 0.39–0.76) as compared with autoantibody-negative patients (OR 0.90, 95% CI 0.41–1.99).

Conclusion. Statistically significant associations were observed between the response to anti-TNF therapy and an RA risk allele at the *PTPRC* gene locus. Additional studies will be required to replicate this finding in additional patient collections.

The long-term outcome in patients with rheumatoid arthritis (RA) is highly dependent on aggressive pharmacologic control of inflammation early in the disease course (1). Despite the importance of selecting the optimal medication soon after disease onset, there is no validated biomarker that can serve as a predictor of drug treatment response, and the biologic mechanism by which some patients fail to respond is incompletely understood. As a consequence, RA patients often develop irreversible joint destruction while their physician searches for an effective drug combination (2). A biomarker would be particularly useful for the assessment of drugs that block the inflammatory cytokine tumor necrosis factor α (TNF α), since these drugs are often used to treat moderate-to-severe RA and yet induce remission in only ~30% of patients (3,4). In addition to tailoring therapy to the appropriate RA patient population, a biomarker of treatment response would provide insight into the drug's mechanism of action and potentially enhance design approaches for more efficient, larger-scale clinical trials for drug development, which ultimately would improve the care of patients with RA.

Several factors, including age, sex, concurrent methotrexate (MTX) therapy, and synovial TNF α expression—but no genetic factors—have been shown to be reliably correlated with the response to anti-TNF therapy (5–8). A major limitation of most genetic studies has been the small sample size, which reduces the power to detect common alleles with a modest effect size. Another limitation is the difficulty in selecting which genetic variants (e.g., single-nucleotide polymorphisms [SNPs]) to test for association. Many pharmacogenetic studies of anti-TNF therapy have focused on SNPs of unknown function within biologically plausible candidate genes.

Recently, substantial progress has been made in understanding the genetic basis for the risk of RA (1,9,10). Much of the success has come from the ability to test comprehensively a large portion of common SNPs in the human genome—genome-wide association stud-

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ies. To date, more than 20 RA risk alleles outside of the major histocompatibility complex (MHC) region (which contains *HLA-DRB1* shared epitope alleles [11]) have been identified and replicated in large collections of autoantibody-positive patients with RA.

Several observations suggest that these same RA risk alleles might also predict the response to anti-TNF therapy. First, many of the RA risk alleles are near genes involved in TNF α signaling, including *PTPRC/CD45*, *TNFAIP3/A20*, *TRAF1*, *TRAF6*, *CD40*, and others (12–17). Because the alleles are associated unambiguously with RA risk, they most likely have functional consequences on nearby genes that are important in RA pathogenesis. Second, RA risk alleles can be used to form subsets of patients according to clinically meaningful categories, most notably, those who have disease-specific autoantibodies (1). By extension, RA risk alleles may also be used to categorize patients into those who respond to anti-TNF therapy and those who do not respond. Finally, risk alleles for RA (e.g., *CTLA4* [18,19]) and other diseases (20,21) are near genes that have been shown to be effective pharmacologic targets. This observation indicates an overlap between the biologic pathways of effective drugs and pathways that influence disease risk.

Based on these observations, we hypothesized that established RA risk alleles are also associated with the response to anti-TNF therapy. To test this hypothesis, we organized an international consortium to study one of the largest available collections of RA patients being treated with anti-TNF therapy.

PATIENTS AND METHODS

Patients. RA patients were selected from 9 different cohorts (as described below). The clinical features of the patients are listed in Table 1. The diagnosis in all patients was defined by satisfaction of the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 criteria for RA (22) or by confirmation from a board-certified rheumatologist. Inclusion criteria for our study were the presence of active disease (defined as a Disease Activity Score in 28 joints [DAS28] ≥ 3.2) prior to initiation of anti-TNF therapy (baseline) and available data on the DAS28 within 3–12 months after the start of the anti-TNF therapy (followup), as well as current treatment with the anti-TNF drug at the followup time point. From each cohort, we collected information on age, sex, disease duration, serotype status (anti-citrullinated protein antibody [ACPA] and/or rheumatoid factor [RF] positive), anti-TNF treatment duration (from start date to followup date), components of the DAS28 at anti-TNF start and followup, and other medications, including disease-modifying antirheumatic drugs (DMARDs) for RA. We defined seropositive (or autoantibody-positive) patients as

those who were RF and/or ACPA positive, and seronegative patients as those who were negative for both (or negative for a single autoantibody, if only one was checked). We restricted our analysis to subjects with a self-reported white European ancestry, if that information was available from the cohort. Informed consent was obtained from each individual, and the institutional review board at each collection site approved the study protocol.

Cohorts. *Autoimmune Biomarkers Collaborative Network (ABCOn).* The ABCOn study is a prospective clinical trial of 116 RA patients who were started on anti-TNF therapy (n = 51 receiving etanercept, n = 22 receiving adalimumab, and n = 43 receiving infliximab). Clinical data were obtained from evaluations at 5 time points: baseline (before therapy) as well as 6 weeks, 12 weeks, 6 months, and 1 year after the start of anti-TNF therapy (23).

Academic Medical Center (AMC) cohort. The AMC study enrolled and prospectively followed up RA patients who received anti-TNF therapy at the Department of Clinical Immunology and Rheumatology at the AMC of the University of Amsterdam. Of these patients, 55 received adalimumab (24) and 102 received infliximab (8). Clinical data obtained included the DAS28 at baseline and after 4, 8, 12, 16, and 24 weeks of treatment. All patients had a DAS28 of ≥ 3.2 at baseline, and the medication was kept stable during the study.

Treatment Strategies for Rheumatoid Arthritis (Behandelstrategieën voor Reumatoïde Artritis [BeSt]) study. The BeSt study is a multicenter, randomized clinical trial of patients with new-onset RA (25). Patients had a high DAS28 at baseline and were assessed every 3 months. Medications were adjusted by a physician based on the DAS28. All patients had a disease duration of < 2 years. All patients were being treated with infliximab at the time that the posttreatment response was measured. We included only a subset of 126 patients whose treatment response, according to the European League Against Rheumatism (EULAR) response criteria, was either a good response or no response.

Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS). BRAGGSS is a prospective multicenter registry of RA patients who receive anti-TNF therapy in the United Kingdom. The treating physician assesses the treatment response at 6-month intervals. More than 1,000 patients have been enrolled to date, and the data have been used in published genetic studies (26,27), but only a subset of 81 patients receiving infliximab and displaying either a EULAR good response or no response were included in the current study. There was no difference in the age, sex, or treatment history among the BRAGGSS patients selected for the current study and the remaining infliximab-treated BRAGGSS patients classified as EULAR good responders or EULAR nonresponders.

Brigham Rheumatoid Arthritis Sequential Study (BRASS). The BRASS is a prospective observational registry of $> 1,000$ RA patients receiving care at the Brigham and Women's Hospital in Boston (28). Patients enrolled in this study include a subset (n = 6) from whom data on the DAS28 (with C-reactive protein [CRP]) were collected at baseline and 12 weeks after the start of anti-TNF therapy, and a subset (n = 49) who were followed up as part of routine care. For the latter, posttreatment disease activity was assessed in 3–12-month intervals following the initiation of anti-TNF therapy. In

Table 1. Characteristics of the 9 clinical cohorts of patients with rheumatoid arthritis receiving anti-TNF therapy*

Study design	ABCoN		AMC		BeSt		BRAGSS		BRASS†		EIRA‡		ERA		KI		JBI	
	Prospective	Prospective	RCT	Observational	Observational	Observational	RCT	Observational	Observational	Observational	RCT	Observational						
Sample size	116	157	126	81	55	291	218	163	76									
Total	47	49	88	42	27	112	68	44	28									
EULAR good response‡	39	77	ND	7	11	104	102	89	33									
EULAR moderate response‡	30	31	38	32	17	75	48	30	15									
EULAR no response‡	All	ada., inflix.	infix.	infix.	All	All	etan.§	All	etan.									
Anti-TNF drug	DAS28-CRP	DAS28-ESR	DAS28-ESR	DAS28-ESR	DAS28-CRP	DAS28-ESR	DAS28-CRP	DAS28-ESR	DAS28-ESR									
Disease activity measure	SIP	SIP	SIP	SIP	Imputed	Imputed	SIP	SIP	SIP									
Genotype method																		
Treatment duration, mean (range) months¶	3.4 (2.4–6.1)	3.7 (1.8–4.2)	(6–12)	6	(3–12)	3.5 (2.1–5)	12 (10–13.5)	3.7 (2.2–5)	3.7									
Age, mean ± SD years	54.6 ± 13.4	54.1 ± 12.8	50.7 ± 13.5	55.2 ± 10.8	56.6 ± 13.0	51.3 ± 12.5	50.6 ± 12.5	55.1 ± 13.5	52.9 ± 10.4									
Sex, % female	79.3	77.7	65.1	72.8	85.5	73.9	72.5	84.7	81.6									
Seropositive status, % ACPA/RF positive	89.8	79.9	81.3	92.3	100	81.8	87.2	85.9	71.1									
Concurrent methotrexate, %	69.1	96.8	97.6	91.4	25.5	74.2	0	100	81.6									
Disease duration, mean ± SD years	10.1 ± 9.7	10.6 ± 13.4	1.4 ± 1.2	11.6 ± 9.7	9.9 ± 9.8	3.3 ± 2.6	1.0 ± 0.9	13.1 ± 10.7	11.4 ± 8.9									

* Anti-TNF = anti-tumor necrosis factor α; ABCoN = Autoimmune Biomarkers Collaborative Network; AMC = Academic Medical Center; BeSt = Treatment Strategies for Rheumatoid Arthritis (Behandelstrategieën voor Reumatoïde Artritis); BRAGSS = Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate; ERA = Immunex Early Rheumatoid Arthritis; KI = Karolinska Institutet; JBI = Jan van Breemen Institute; RCT = randomized controlled trial; ND = not determined (due to exclusion of moderate responders); ada. = adalimumab; infix. = infliximab; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; SIP = Sequenom iPLEX; ACPA = anti-citrullinated protein antibody; RF = rheumatoid factor.

† Data on the Brigham Rheumatoid Arthritis Sequential Study (BRASS) cohort were imputed from Affymetrix 6.0 genome-wide single-nucleotide polymorphism (SNP) data, while data on the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study cohort were imputed from Illumina 317K genome-wide SNP data.

‡ The European League Against Rheumatism (EULAR) response categories were as follows: good response = improvement in the Disease Activity in 28 joints (DAS28) of ≥1.2 and a followup DAS28 of ≤3.2; moderate response = all values between good response and no response; no response = improvement in the DAS28 of ≤0.6, or improvement in the DAS28 of >0.6 but <1.2, and a followup DAS of ≥5.1.

§ Treatment with etanercept (etan.) consisted of either 10 mg or 25 mg administered twice weekly.

¶ For those studies in which mean values are missing, start dates were not available. For those studies in which ranges are missing, data were assessed at a single time point.

total, 24 patients were receiving etanercept, 26 were receiving adalimumab, and 5 were receiving infliximab.

Epidemiological Investigation of Rheumatoid Arthritis (EIRA). The EIRA is a population-based study of incident RA that was initiated in 1996. Clinical followup data are registered in the Swedish Rheumatology and Biologics Register by the treating rheumatologist at each visit as part of a national surveillance system (29). Patients enrolled in the present study are a subset of those included in the EIRA study, comprising those patients who started anti-TNF therapy as the first biologic treatment during the followup period, with the DAS28 determined at baseline and at the 3-month followup visit ($n = 100$ receiving etanercept, $n = 144$ receiving infliximab, and $n = 47$ receiving adalimumab).

Immunex Early Rheumatoid Arthritis (ERA) study. The ERA study was a randomized clinical trial of 632 patients with early RA treated for 12 months with either etanercept (10 mg or 25 mg subcutaneously twice per week) or MTX (orally once per week) (30). More than 80% of patients continued receiving etanercept for 12 months, at which time disease activity and treatment response were assessed. At baseline, the 632 RA patients shared similar levels of disease activity and had RA for no more than 3 years. A subset of the patients consented to undergo genetic studies of treatment response ($n = 457$). Of these, 218 completed 12 months of etanercept treatment ($n = 106$ receiving 10 mg and $n = 112$ receiving 25 mg) and had a sufficient quantity of DNA, after whole-genome amplification, for direct genotyping. These samples have been used in a candidate gene study of treatment response (31).

Karolinska Institutet (KI) study. The KI study cohort consisted of RA patients from the outpatient clinics within the Department of Rheumatology at Karolinska University Hospital in Stockholm (32). The inclusion criteria included having received anti-TNF therapy as the first biologic treatment during 1999–2007, having provided a DNA sample to the Rheumatology Biobank, and having clinical followup data available as part of the Swedish Rheumatology and Biologics Register (as described above for the EIRA), including the DAS28 at baseline and at the 3-month followup visit. Patients were excluded if they were already enrolled in the EIRA. From a total of 632 RA patients in this group, 486 had clinical followup data available, and of these, 163 provided a DNA sample to the Rheumatology Biobank ($n = 124$ receiving infliximab, $n = 31$ receiving etanercept, and $n = 8$ receiving adalimumab). The baseline characteristics of those patients with an available DNA sample were similar to those who had not provided DNA.

Jan van Breemen Institute (JBI) study. Since 2005, all Dutch patients with RA starting treatment with etanercept have been enrolled in a cohort study at the JBI in Amsterdam. RA patients are enrolled in the study if they are eligible for anti-TNF therapy, in accordance with the Dutch consensus statement on TNF-blocking therapy. Eligibility criteria include the presence of active disease (DAS28 ≥ 3.2) and having failed treatment with at least 2 DMARDs, including MTX at the maximal or tolerable dosage. Exclusion criteria are active infection and pregnancy. Enrollment in the JBI cohort is still open, and the current genetic study includes the first 76 consecutive RA patients for whom genetic and clinical data were available. Disease activity (according to the DAS28) was assessed at baseline and after 4, 16, and 28 weeks of therapy.

All patients were treated with etanercept at a dose of 50 mg subcutaneously every week or 25 mg twice a week.

Definition of treatment response. The DAS28 was calculated directly from individual patient data, on the basis of the number of swollen and tender joints, the level of acute-phase response (using either the erythrocyte sedimentation rate or level of CRP), and patient's general health assessment (33). Table 1 lists the specific DAS28 versions used in each of the cohorts. Our primary analysis was to categorize patients according to the EULAR response criteria (34), in which a good response was defined as a followup DAS28 of < 3.2 and improvement in the DAS28 of > 1.2 from baseline, while nonresponse was defined as improvement in the DAS28 of < 0.6 from baseline or improvement of ≤ 1.2 and a followup DAS28 of > 5.1 ; moderate response was defined as those DAS28 values in between. In a secondary analysis, we used the change in DAS28 (Δ DAS28) from baseline to the time that treatment response was assessed (between 3 months and 12 months posttreatment).

Clinical factors. We tested clinical factors for any association with treatment response (according to the EULAR response criteria), using logistic regression. Clinical factors included age, sex, disease duration, treatment duration, RF and ACPA status, concomitant medications, and the DAS28 prior to treatment. We also tested for a cohort effect in the logistic model by creating dummy variables for each cohort.

Genotyping. We selected 31 validated or highly suggestive MHC and non-MHC RA risk alleles from recent large-scale genetic studies (12–19,35–42). Tag SNPs for the MHC were selected from a high-density SNP genotyping study across the MHC (42,43) and from the best independent tag SNPs in an RA genome-wide association study (14). Only 5 of the 9 cohorts had 4-digit *HLA-DRB1* genotype data (the ABCoN, BeSt, BRAGGSS, EIRA, and ERA studies). To assess population stratification, we genotyped 3 SNPs with highly differentiated allele frequencies across individuals of European ancestry (44) (for a complete list of the SNPs genotyped, see Supplementary Table 1, available on the *Arthritis & Rheumatism* Web site at <http://www3.interscience.wiley.com/journal/76509746/home>). As shown in Table 1, genotype data were imputed from Affymetrix 6.0 (900K) data (for those patients in the BRASS cohort) or from Illumina 317K data (for those patients in the EIRA cohort), as previously described (12). Although IMPUTE provides probability scores (45), we used integer allele counts, since there is little difference between probability scores and counts (results not shown). For the remaining cohorts, we genotyped 31 SNPs (as well as proxy SNPs and European ancestry informative markers [AIMs] [44]) using Sequenom iPLEX as previously described (12), which was performed at the Broad Institute. Within each cohort, we removed individuals with $> 10\%$ missing genotypes (based on all available genotype data) and we removed SNPs that had $> 5\%$ missing genotype data, that had a minor allele frequency of $< 1\%$, and that, on testing for Hardy-Weinberg equilibrium, were found to be significantly different ($P < 0.001$).

Statistical analysis. In our primary analysis, we tested each SNP for an association with the anti-TNF response (EULAR good response versus no response) using logistic regression, assuming a log additive model. We controlled for age, sex, concurrent treatment with MTX, and cohort effect, as

Table 2. Correlations of clinical characteristics with treatment response according to the 3 EULAR response categories among 1,283 patients with rheumatoid arthritis treated with anti-tumor necrosis factor α therapy*

	Good response (n = 505)	Moderate response (n = 462)	No response (n = 316)	<i>P</i>
Sex, % female	71	77	84	<0.0001
Age, mean \pm SD years	51.5 \pm 12.8	54.0 \pm 12.3	53.3 \pm 13.2	0.006
Disease duration, mean \pm SD years	6.5 \pm 9.9	6.9 \pm 8.4	6.6 \pm 8.8	0.71
RF positive, %	79	81	76	0.19
ACPA positive, %	77	77	74	0.77
Concurrent methotrexate, %	73	66	67	0.07
Concurrent steroid, %	33	39	35	0.21
Concurrent NSAIDs, %	57	60	55	0.45
Treatment duration, mean \pm SD months	5.2 \pm 3.4	5.6 \pm 3.6	5.3 \pm 3.5	0.29
DAS28, mean \pm SD				
Baseline	5.4 \pm 1.0	5.9 \pm 1.1	5.3 \pm 1.3	<0.0001
Followup	2.3 \pm 0.6	4.1 \pm 0.8	5.2 \pm 1.2	<0.0001

* Analysis of variance was used to compare age, disease duration, and baseline and followup Disease Activity Score in 28 joints (DAS28), while the chi-square test was used to compare sex distribution, rheumatoid factor (RF)/anti-citrullinated protein antibody (ACPA) status, and concurrent medications among the 3 European League Against Rheumatism (EULAR) categories of response. NSAIDs = nonsteroidal antiinflammatory drugs.

well as for the DAS28 at start of anti-TNF therapy, in a multivariate model that included SNPs potentially predictive of the anti-TNF response. In our secondary analysis, we modeled SNPs potentially predictive of the Δ DAS28, using univariate and multivariate linear regression models in which we adjusted for age, sex, concurrent treatment with MTX, cohort effect, and the DAS28 at start of anti-TNF therapy. We made no assumptions about the direction of effect on treatment response with respect to the risk of RA. We considered a Bonferroni-corrected *P* value of less than 0.05 as statistically significant, which, in our study of 31 SNPs, corresponded to *P* < 0.0016.

For each SNP, we tested for heterogeneity across the 9 cohorts using H statistics. We performed stratified analyses according to clinical characteristics to explore sources of heterogeneity. To compare the association among subgroups of RA patients for our primary outcome, we used a logistic regression model that included genotype, clinical category, and genotype \times clinical category; the reported *P* value indicates the significance of the interaction term, which compares the odds ratio (OR) and 95% confidence interval (95% CI) for each clinical category. For our secondary outcome, the Δ DAS28, we used a general linear model that included genotype, clinical category, and genotype \times clinical category, as well as the DAS28 at the start of anti-TNF therapy; the reported *P* value again indicates the significance of the interaction term, which, in this analysis, compares the beta values within each clinical category.

RESULTS

Patient characteristics. The clinical characteristics of the 1,283 RA patients with active disease from our 9 cohorts are shown in Table 1. Patients were started on infliximab (n = 625), etanercept (n = 502), or adalimumab (n = 156), and most were treated concurrently with MTX (n = 955 [74.4%]). All patients were receiv-

ing anti-TNF therapy at the time that treatment response was assessed. Three of the cohorts comprised patients with early-onset RA (BeSt, EIRA, and ERA), while 2 of the studies were randomized controlled trials (BeSt and ERA). The percentage of patients who were seropositive for either RF or ACPAs was similar among the 9 cohorts (71.1–100%).

To build a multivariate clinical model for our genetic association study, we first tested for associations between each clinical variable and the EULAR response classification (good response, moderate response, or no response). As shown in Table 2, younger age (*P* = 0.006) and male sex (*P* < 0.0001) were significantly correlated with better outcome. Concurrent treatment with MTX demonstrated a trend toward significance, with 73% of the patients treated concurrently with MTX classified as having a EULAR good response, as compared with 66% and 67% classified as EULAR moderate responders and EULAR nonresponders, respectively (each *P* = 0.07). The pretreatment (baseline) DAS28 was significantly correlated with treatment response, but the trend was not linear across the 3 EULAR categories (e.g., those in the moderate response category had the highest baseline DAS28, at a mean of 5.9). Based on these results, we included age, sex, concurrent treatment with MTX, baseline DAS28, and a cohort variable into the multivariate model.

Genetic associations. We tested 31 RA risk alleles for associations with the response to anti-TNF therapy, using univariate and multivariate logistic regression analyses. The primary outcome was a EULAR good response versus no response (n = 505 versus n =

Table 3. Associations of 31 SNPs with EULAR good response versus no response in univariate and multivariate models*

chr	SNP	Gene	POS (bp)	MAF	Univariate		Multivariate†	
					OR	<i>P</i>	OR	<i>P</i>
1	rs10919563	<i>PTPRC</i>	196,967,065	0.12	0.59	0.0004	0.55	0.0001
1	rs11586238	<i>CD58</i>	117,064,661	0.25	1.31	0.04	1.32	0.04
3	rs4535211	<i>PLCL2</i>	17,048,001	0.44	0.83	0.07	0.84	0.11
7	rs11761231	<i>PODXL</i>	131,020,579	0.36	0.84	0.14	0.86	0.23
6	rs1341239	<i>PRL</i>	22,412,183	0.37	1.17	0.16	1.21	0.09
11	rs540386	<i>TRAF6</i>	36,481,869	0.13	0.79	0.16	0.81	0.23
10	rs2104286	<i>IL2RA</i>	6,139,051	0.24	0.85	0.21	0.84	0.18
6	rs6920220	<i>TNFAIP3</i>	138,048,197	0.23	0.87	0.30	0.87	0.32
6	rs4895501	<i>TNFAIP3</i>	138,329,253	0.39	1.11	0.32	1.11	0.33
1	rs2476601	<i>PTPN22</i>	114,179,091	0.16	1.14	0.35	1.15	0.32
1	rs3890745	<i>TNFRSF14</i>	2,543,484	0.31	1.11	0.35	1.13	0.30
6	rs548234	<i>PRDMI</i>	106,674,727	0.33	0.91	0.37	0.89	0.30
9	rs2812378	<i>CCL21</i>	34,700,260	0.38	1.09	0.40	1.06	0.57
2	rs13031237	<i>REL</i>	60,989,633	0.37	0.92	0.43	0.94	0.56
20	rs4810485	<i>CD40</i>	44,181,354	0.21	0.91	0.46	0.94	0.66
10	rs4750316	<i>PRKCQ</i>	6,433,266	0.18	0.91	0.50	0.92	0.54
2	rs3087243	<i>CTLA4</i>	204,447,164	0.39	1.07	0.52	1.13	0.26
6	rs4947332	<i>HLA*0101</i>	31,942,176	0.04	1.16	0.62	1.06	0.85
4	rs231707	<i>TNIP2</i>	2,664,183	0.19	0.93	0.64	0.89	0.46
9	rs3761847	<i>TRAF1-C5</i>	122,730,060	0.45	1.04	0.67	1.05	0.66
6	rs6457617	<i>DR4</i>	32,771,829	0.33	0.96	0.72	0.94	0.57
2	rs1980421	<i>CD28</i>	204,318,249	0.23	1.04	0.74	1.04	0.74
4	rs6822844	<i>IL2-IL21</i>	123,728,871	0.14	1.05	0.75	1.06	0.70
8	rs13277113	<i>BLK</i>	11,386,595	0.29	1.04	0.76	0.99	0.95
6	rs3817964	<i>HLA*0401</i>	32,475,975	0.08	0.95	0.77	0.98	0.91
22	rs3218253	<i>IL2RB</i>	35,874,756	0.27	1.04	0.78	1.09	0.51
6	rs394581	<i>TAGAP</i>	159,402,509	0.26	1.03	0.79	1.04	0.71
6	rs2621377	<i>HLA*DPB1</i>	32,871,088	0.39	1.02	0.83	1.04	0.73
6	rs13207033	<i>TNFAIP3</i>	138,007,111	0.23	1.01	0.92	0.99	0.96
7	rs42041	<i>CDK6</i>	92,084,680	0.26	1.00	0.98	0.97	0.83
2	rs11889341	<i>STAT4</i>	191,651,987	0.23	1.00	1.00	1.03	0.85

* For analyses of association with the European League Against Rheumatism (EULAR) response categories (good response versus no response), results are rank-ordered by *P* value. Information about each single-nucleotide polymorphism (SNP) is shown, including chromosome (chr), SNP name, nearest gene, and chromosome position (POS). The minor allele frequency (MAF) is calculated from all 1,283 rheumatoid arthritis (RA) samples. The odds ratio (OR) and *P* values are shown for univariate and multivariate analyses, where the ORs are with respect to the minor allele.

† The multivariate model was adjusted for sex, age, concurrent treatment with methotrexate, the Disease Activity Score in 28 joints at baseline, and cohort effect.

316, respectively). We chose this dichotomous outcome measure to minimize heterogeneity across the 9 cohorts, since the DAS28 is more accurate for patients with either high or low disease activity (46).

As shown in Table 3, only a single SNP (rs10919563) showed a significant association ($P < 0.01$) with a EULAR good response to anti-TNF therapy. This SNP is in the *PTPRC* gene (also known as *CD45*), and was significantly associated with treatment response in both univariate and multivariate models ($P = 0.0004$ and $P = 0.0001$, respectively). The major allele (G allele), which is a known predictor of RA risk, is the same allele that was found to be a predictor of favorable response; in the presence of the major allele, the OR for the

likelihood of a EULAR good response was 0.59 and 0.55 in univariate and multivariate models, respectively. When the data were corrected for multiple hypothesis testing, taking into account the number of SNPs tested (calculated as the *P* value divided by the number of SNPs, or 0.05/31), the association remained significant ($P = 0.0016$) (for more detailed data on all 31 SNPs tested in each of the 9 cohorts, see Supplementary Table 2, available on the *Arthritis & Rheumatism* Web site at <http://www3.interscience.wiley.com/journal/76509746/home>).

To determine whether the *PTPRC* SNP or any other SNP was associated with the secondary outcome measure, the Δ DAS28, as a continuous variable, we

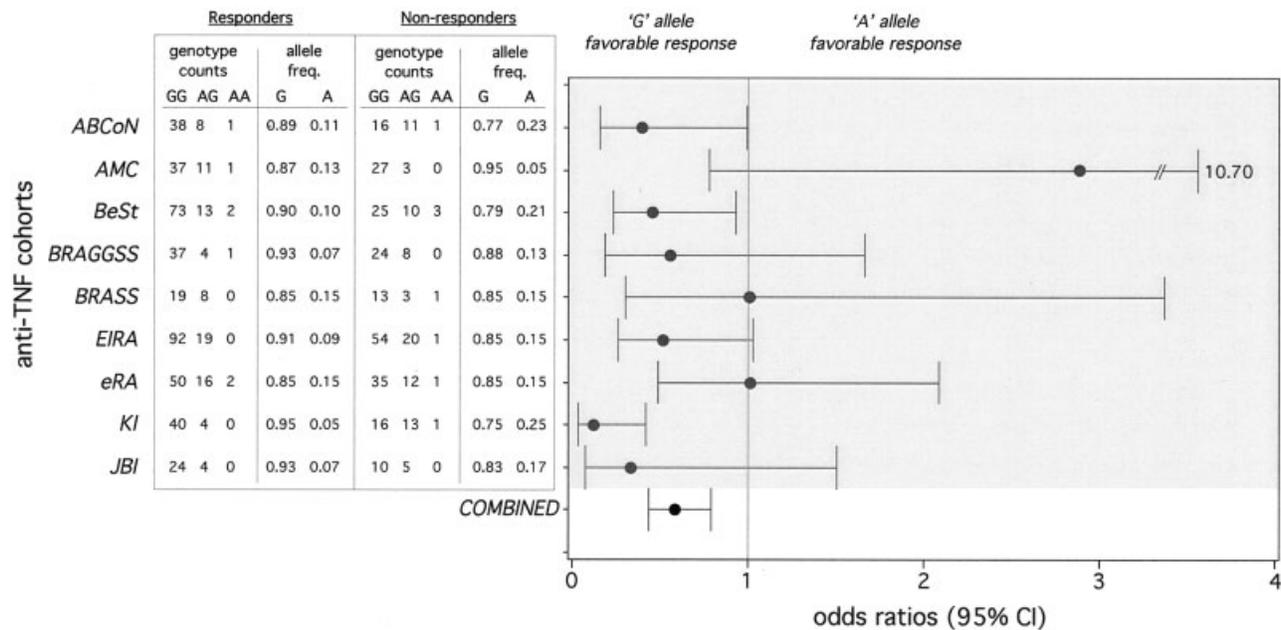


Figure 1. Primary end point analysis. Shown are genotype counts and allele frequencies (freq.) in patients with rheumatoid arthritis (RA) according to European League Against Rheumatism (EULAR) treatment response (good response versus no response) to anti-tumor necrosis factor α (anti-TNF) therapy in each of the 9 different cohorts (left), and a forest plot of the results of association analyses of *PTPRC* in relation to a favorable EULAR response (right). The major allele (G allele) of *PTPRC* is the RA risk allele, and as the forest plot shows for each cohort and for the combined analysis ($n = 821$ patients), the same G allele is associated with a favorable response to anti-TNF therapy. The odds ratio (OR) point estimates and 95% confidence intervals (95% CIs) are shown for the major G allele relative to the minor A allele. For purposes of scale, the upper bound of the 95% CI for the Academic Medical Center (AMC) cohort is not shown (95% CI 0.78–10.70). ABCOn = Autoimmune Biomarkers Collaborative Network; BeSt = Treatment Strategies for Rheumatoid Arthritis (Behandelstrategieën voor Reumatoïde Artritis); BRAGGSS = Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate; BRASS = Brigham Rheumatoid Arthritis Sequential Study; EIRA = Epidemiological Investigation of Rheumatoid Arthritis; eRA = Immunex Early Rheumatoid Arthritis; KI = Karolinska Institutet; JBI = Jan van Breemen Institute.

tested each SNP using univariate and multivariate linear regression models. This analysis included an additional 462 RA patients classified as having a EULAR moderate response, for a total of 1,283 RA patients who were started on anti-TNF therapy and evaluated for treatment response. Consistent with a true-positive result, the *PTPRC* SNP remained significantly associated with a favorable anti-TNF response ($P = 0.0005$ and $P = 0.0002$ in univariate and multivariate models, respectively). No other SNP was found to be significantly associated at $P < 0.01$, in either univariate or multivariate analyses (for detailed results on the association of each of the 31 SNPs with the Δ DAS28 in the 9 cohorts, see Supplementary Tables 3 and 4, available on the *Arthritis & Rheumatism* Web site at <http://www3.interscience.wiley.com/journal/76509746/home>).

We next examined whether the *PTPRC* SNP was correlated with treatment response across all 9 cohorts, or whether the correlation was specific to a single cohort

(or subset of cohorts). As shown in Figure 1, the OR for a favorable treatment response (EULAR good response versus no response) was relatively consistent across all cohorts. The KI cohort had the smallest point estimate of response (OR 0.12, 95% CI 0.04–0.42), whereas the point estimate for the AMC cohort was the highest (OR 2.88, 95% CI 0.78–10.7).

A formal test for heterogeneity among cohorts showed a nonsignificant trend across all cohorts ($P = 0.06$). After removal of the AMC cohort, heterogeneity was reduced ($P = 0.19$) and the association with *PTPRC* became more significant ($P = 0.00003$). After removal of the KI cohort, both heterogeneity and the *PTPRC* association became less significant ($P = 0.21$ and $P = 0.0097$, respectively). When we tested for heterogeneity in our secondary analysis of an association with the Δ DAS28 as a continuous trait, we observed modest evidence of differences across the 9 cohorts ($P = 0.006$) (Figure 2). (Results of tests for heterogeneity of associ-

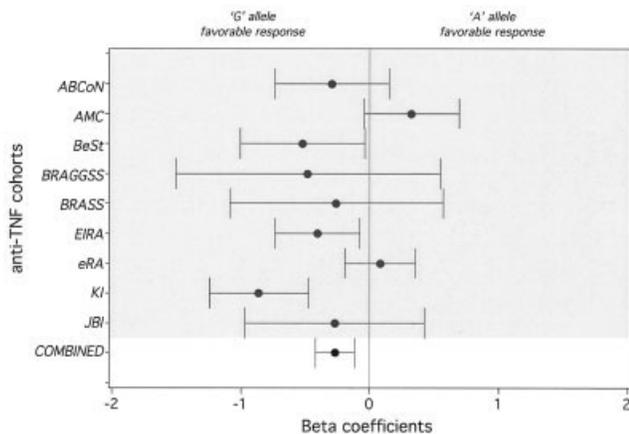


Figure 2. Secondary end point analysis. The forest plot shows the results of the association analyses of *PTPRC* (major G allele and minor A allele) in relation to change in the Disease Activity Score in 28 joints from baseline to followup, as a quantitative trait, in patients with rheumatoid arthritis treated with anti-TNF therapy. The beta coefficient point estimates and 95% confidence intervals are shown for each cohort as well as for the combined analysis ($n = 1,283$ patients). See Figure 1 for definitions.

ations with all 31 SNPs are listed in Supplementary Table 5, available on the *Arthritis & Rheumatism* Web site at <http://www3.interscience.wiley.com/journal/76509746/home>.

To determine whether the *PTPRC* SNP was associated with treatment response in a specific group of RA patients or in relation to a specific anti-TNF drug, we stratified the primary and secondary analyses by potentially important clinical categories, as follows: the RF and ACPA autoantibody status, the anti-TNF drug class, study design, sex, concurrent treatment with MTX, and disease duration (Table 4). The association was stronger among 1,037 seropositive patients with RA (defined as being either RF+ or ACPA+) than among 195 seronegative patients with RA. However, the ORs for an association of the *PTPRC* SNP with favorable treatment response were not statistically significantly different ($P = 0.26$) between seropositive and seronegative patients with RA (seropositive, OR 0.55, 95% CI 0.39–0.76; seronegative, OR 0.90, 95% CI 0.41–1.99). Similarly, a suggestive trend toward an association with serologic status was observed when the Δ DAS28 was used as the end point (Table 4).

When we restricted our analyses of the *PTPRC* SNP to the subcategory of seropositive patients with RA, we found less evidence of heterogeneity in the associations across the cohorts (heterogeneity in association with a EULAR good response versus no response, $P =$

0.11; heterogeneity in association with the Δ DAS28, $P = 0.02$). The association with the *PTPRC* SNP was also strongest within those subgroups that had the largest number of patients (e.g., infliximab-treated patients [$n = 625$], observational study design [$n = 666$], female sex [$n = 975$], and concurrent treatment with MTX [$n = 955$]). However, except for the study design variable in the analysis of association with the Δ DAS28, none of the clinical categories showed an OR that reached a convincing level of statistical significance ($P < 0.05$) when any other SNP was assessed (see Supplementary Table 6 [stratified by serologic status] and Supplementary Table 7 [stratified by anti-TNF drug] for detailed results on all of the RA risk SNPs, available on the *Arthritis & Rheumatism* Web site at <http://www3.interscience.wiley.com/journal/76509746/home>).

Finally, we assessed whether population stratification might account for our findings, despite the fact that the RA patients self-reported almost exclusively white European ancestry. We genotyped 3 AIMs and tested for an association with treatment outcome (see Supplementary Figure 1, available on the *Arthritis & Rheumatism* Web site at <http://www3.interscience.wiley.com/journal/76509746/home>). Only 1 of the 3 AIMs, rs7696175 (*TLR1* locus), showed an association with our primary end point, a EULAR good response versus no response ($P = 0.001$), and with our secondary end point, the Δ DAS28 ($P = 0.002$). However, there was no correlation between the *PTPRC* SNP association and the *TLR1* AIM association; the frequency of the *PTPRC* allele was similar across the 3 *TLR1* genotype classes. The cohort demonstrating the strongest association between the *PTPRC* SNP and treatment response (the KI cohort; OR 0.12) showed no association between the *TLR1* AIM and treatment response. Moreover, inclusion of the *TLR1* SNP in our multivariate model did not change the association of treatment response with the *PTPRC* SNP (results not shown). When we limited our analysis to seropositive patients with RA, we found only modest evidence of an association of treatment response with the *TLR1* AIM ($P = 0.01$), although there was continued evidence of an association with the *PTPRC* locus ($P = 0.0003$ for the association with the primary end point).

DISCUSSION

In this study, we have identified an RA risk allele, rs10919563 in the *PTPRC/CD45* gene, that is also associated with the response to anti-TNF therapy in a large collection of RA patients. The association was found to

Table 4. Stratified analyses of interactive associations of the *PTPRC* single-nucleotide polymorphism (rs10919563) with a EULAR good response and with the Δ DAS28*

Clinical category	No. of patients	EULAR categories					Δ DAS28				
		OR	Lower CI	Upper CI	<i>P</i> for association	<i>P</i> between subgroups†	Beta	Lower CI	Upper CI	<i>P</i> for association	<i>P</i> between subgroups†
ACPA/RF autoantibody status						0.26					0.14
Seropositive	1,037	0.55	0.39	0.76	0.0003		0.30	0.14	0.46	0.0002	
Seronegative	195	0.90	0.41	1.99	0.79		-0.03	-0.48	0.42	0.89	
Anti-TNF drug						0.66					0.61
Infliximab	625	0.56	0.37	0.84	0.005		0.35	0.11	0.59	0.005	
Etanercept	502	0.55	0.34	0.91	0.02		0.22	0.002	0.43	0.05	
Adalimumab	156	0.86	0.36	2.09	0.74		0.16	-0.24	0.55	0.44	
Study design						0.21					0.01
Prospective	273	0.86	0.44	1.68	0.65		-0.04	-0.33	0.24	0.77	
RCT	344	0.67	0.41	1.10	0.11		0.17	-0.10	0.43	0.22	
Observational	666	0.44	0.28	0.68	0.0003		0.51	0.28	0.75	<0.0001	
Sex						0.77					0.89
Female	975	0.57	0.41	0.81	0.001		0.26	0.08	0.43	0.004	
Male	308	0.63	0.34	1.18	0.15		0.29	-0.01	0.59	0.06	
Concurrent MTX						0.24					0.20
Yes	880	0.52	0.37	0.75	0.0004		0.34	0.14	0.54	0.0006	
No	397	0.76	0.45	1.29	0.31		0.12	-0.12	0.36	0.31	
Disease duration by cohort						0.67					0.78
Early-onset cohort	635	0.62	0.42	0.93	0.02		0.25	0.05	0.46	0.02	
Other cohorts	648	0.55	0.35	0.85	0.007		0.29	0.07	0.52	0.01	
Disease duration by years since onset						0.73					0.70
Early onset (<2 years)	499	0.62	0.39	0.99	0.05		0.24	-0.002	0.48	0.05	
Not early onset	784	0.56	0.38	0.82	0.003		0.29	0.10	0.49	0.004	

* Stratified analyses of rheumatoid arthritis (RA) patients receiving anti-tumor necrosis factor α (anti-TNF) therapy were performed for clinically relevant traits. Results are shown for the primary outcome, the European League Against Rheumatism (EULAR) response category of good response (versus no response), and the secondary outcome, change in the Disease Activity Score in 28 joints (Δ DAS28) from baseline, as a quantitative trait. Associations with the primary outcome are shown as the odds ratio (OR) with lower and upper bounds of the 95% confidence interval (95% CI), while associations with the secondary outcome are shown as the beta coefficient with lower and upper 95% CI. Seropositive is defined as positive serologic status for either anti-citrullinated protein autoantibodies (ACPA) or rheumatoid factor (RF). RCT = randomized controlled trial; MTX = methotrexate.

† For comparison of the association with the primary outcome (EULAR categories) among subgroups of RA patients, a logistic regression model was used, which included the genotype, clinical category, and genotype \times clinical category. For comparison of the association with the secondary outcome, Δ DAS28, a general linear model was used, which included genotype, clinical category, and genotype \times clinical category, as well as baseline DAS28. The reported *P* value between subgroups is for the interaction term.

be strongest among those patients who were seropositive for either RF or ACPA autoantibodies.

The statistical evidence in favor of a true-positive result indicating an association with the *PTPRC* SNP is very strong ($P = 0.0001$ in our multivariate model of EULAR good response versus no response), but not to the point where this finding can be classified as an unambiguous genetic biomarker of treatment response. In this study, we adjusted for multiple hypothesis testing according to the number of SNPs tested ($n = 31$), which yielded a *P* value of less than 0.0016, indicating statistical significance. The finding of an association at the *PTPRC* gene locus clearly surpasses this level of significance when assessed in relation to our primary and secondary end points. Nonetheless, independent replication is re-

quired to confirm definitively that the *PTPRC* SNP is associated with response to anti-TNF therapy.

There are important sources of heterogeneity that may confound our results. We combined the results across 9 different cohorts, each of which is quite different with regard to study design, ascertainment criteria, and duration of followup (as well as other clinical factors, as shown in Table 1). We chose to balance the gain in power achieved by the increase in sample size by the potential bias introduced through unmeasured confounding variables. To minimize any bias, we developed a clinical prediction model (Table 2) and restricted our primary analysis to the 2 most dichotomous EULAR categories of response. For our *PTPRC* finding, we found an association in autoantibody-positive ($P =$

0.0003), but not autoantibody-negative, patients, although the difference between the 2 categories was not statistically significant (Table 4).

Another potential source of heterogeneity is population ancestry. Although we did not have access to detailed ethnic information on all of the patients, and we do not yet have access to genome-wide SNP genotype data to match subjects using genetic data, the majority of patients from these collections are of European ancestry. In addition, we observed that the association with *PTPRC* stood out from the other 31 SNPs, as well from 3 SNPs that are highly differentiated across European populations (see Supplementary Figure 1 on the *Arthritis & Rheumatism* Web site at <http://www3.interscience.wiley.com/journal/76509746/home>). Taken together, these results suggest that population stratification alone does not account for the *PTPRC* result.

If the *PTPRC* association is confirmed in additional RA patient collections, then one of the most important applications will be in providing biologic insight into the mechanism by which some patients respond to treatment and others do not. The SNP associated with both RA and response to anti-TNF therapy lies within the *PTPRC* gene (also known as *CD45*). *PTPRC*, which is a transmembrane receptor-like molecule specifically expressed on the cell surface of all nucleated hematopoietic cells, is an essential regulator of T and B cell antigen receptor signaling (47) and a mediator of TNF α secretion from monocytes (48). Elegant studies in the mouse and human have demonstrated that *PTPRC* affects cellular responses by controlling the relative threshold of sensitivity to external stimuli, including secreted cytokines (49).

The application of our *PTPRC* finding to the clinical care of RA patients is not yet known. Given the modest effect on treatment response (OR of 0.55 in a multivariate model), this finding alone will likely have little impact on determining which patients should receive anti-TNF therapy. As additional genetic variants are identified, a composite genetic prediction score might provide sufficient discrimination between responders and nonresponders to be clinically useful. In addition, it will be important to test whether the *PTPRC* allele is associated with response to other medications used to treat RA, or whether the association is specific to anti-TNF therapy.

There are several strengths in our study. First, we have assembled one of the largest collections of RA patients treated with anti-TNF therapy available to date for pharmacogenetic studies. The largest previous anti-TNF pharmacogenetic study was in 1,070 RA patients

(27). Our sample size improves the power to detect common variants of moderate effect size (OR >1.5, such as that observed for the *PTPRC* variant in our study), but is still underpowered to detect more modest effects, such as observed for most known RA risk loci outside of the MHC region. Second, we focused our primary analysis on more extreme response categories—EULAR good response versus no response, excluding moderate response patients—rather than on all 3 EULAR categories or Δ DAS28 as a quantitative trait. A dichotomous approach has conceptual appeal, given that the DAS28 is more accurate for patients with either high or low disease activity (46). Whether this approach truly improves power in RA pharmacogenetic studies will ultimately require additional true-positive associations for empirical comparison. Third, we have focused on SNPs with a higher prior probability of being functional and important in RA pathogenesis, the RA risk alleles. This approach facilitates interpretation of statistical significance and biologic plausibility.

Although we have strong statistical evidence to support an association with one RA risk allele (rs10919563 in the *PTPRC* gene), our study also demonstrates that the majority of RA risk alleles do not appear to be associated with treatment response. This finding is consistent with that of a recent study from the BRAGGSS, which showed no evidence for association between *PTPN22* or *HLA-DRB1* shared epitope alleles and treatment response (26). (Of note, only 5 of the 9 cohorts had 4-digit *HLA-DRB1* genotype data available, and therefore we were unable to test whether shared epitope alleles are associated with treatment response in our study.) It is possible that RA risk alleles have much more modest effects on response to anti-TNF therapy, and that our study is underpowered to detect these effects. However, we failed to see any trend toward significance based on directionality of the effect on RA risk and response to anti-TNF therapy (see Supplementary Tables 2 and 3 on the *Arthritis & Rheumatism* Web site at <http://www3.interscience.wiley.com/journal/76509746/home>). Alternatively, alleles associated with response to therapy may be different from those that are associated with disease risk.

In conclusion, we present strong statistical evidence in favor of a true-positive association between a SNP in the *PTPRC* gene and response to anti-TNF therapy in RA patients, especially among those seropositive for either ACPAs or RF autoantibodies. If additional studies confirm our findings, this will guide functional studies to understand how the *PTPRC* genetic variant provides biologic insight into why some patients

respond to anti-TNF therapy while others do not. In addition, it is also clear that an unbiased scan of the human genome will be required to identify novel genetic factors associated with response to anti-TNF therapy.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Plenge had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Cui, Saevarsdotir, Padyukov, de Vries, Raychaudhuri, Alfredsson, Wolbink, van der Horst-Bruinsma, Weinblatt, Shadick, Wilson, Isaacs, Seldin, Tak, Klareskog, Gregersen, Karlson, Plenge.

Acquisition of data. Cui, Saevarsdotir, Thomson, Padyukov, van der Helm-van Mil, Nititham, Hughes, de Vries, Alfredsson, Askling, Wedrén, Guiducci, Wolbink, Crusius, van der Horst-Bruinsma, Herenius, Weinblatt, Shadick, Worthington, Batliwalla, Kern, Morgan, Wilson, Hyrich, Seldin, Moreland, Behrens, Allaart, Criswell, Huizinga, Bridges, Toes, Barton, Klareskog, Gregersen, Plenge.

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