

Genetic markers associated with clinical and radiographic response in adalimumab plus methotrexate- or methotrexate-treated rheumatoid arthritis patients in OPTIMA

A. Skapenko¹, J.S. Smolen², A. Kavanaugh³, V. Arora⁴, H. Kupper⁵, H. Schulze-Koops¹

¹Division of Rheumatology and Clinical Immunology, Med. Klinik und Poliklinik IV, University of Munich, Germany; ²Medical University of Vienna and Hietzing Hospital, Vienna, Austria; ³University of California, San Diego, La Jolla, CA, USA; ⁴AbbVie Inc, North Chicago, IL, USA; ⁵AbbVie Deutschland GmbH & Co KG, Ludwigshafen, Germany.

Abstract

Objective

Biologics, including tumour necrosis factor inhibitors such as adalimumab (ADA), have significantly improved outcomes in rheumatoid arthritis (RA). Because the clinical course of RA and response to therapy may be influenced by the genetic background of the patient, the objective of this retrospective parallel-assigned case-control analysis was to evaluate the associations between candidate genetic markers for RA with clinical and radiographic responses to ADA + methotrexate (MTX) or MTX monotherapy in the Optimal Protocol for Treatment Initiation with MTX and ADA (OPTIMA) study.

Methods

Three candidate genetic markers were tested: HLA-DRB1 shared epitope (SE), interleukin 4 receptor (IL4R) single nucleotide polymorphism (SNP) rs1805010, and Fc gamma receptor IIb (FcγRIIb) SNP rs1050501. Genetic associations with week 26 clinical and radiographic responses during treatment with ADA + MTX or MTX monotherapy were assessed using summary statistics, chi-square or Fisher's exact test, correlation, regression models, and corrected for multiple-comparisons.

Results

Low disease activity ($p=0.008$) and improvement in American College of Rheumatology 20%, 50% and 70% response criteria ($p=0.02$, 0.01 , and 0.02 , respectively) were associated with HLA-DRB1 SE copy numbers in the ADA + MTX treatment arm, and the FcγRIIb SNP was a predictor of remission. The IL4R SNP correlated with radiographic progression in patients receiving MTX monotherapy, supporting previous findings.

Conclusion

This pharmacogenetic analysis identified genetic components that contribute to clinical responses to anti-rheumatic therapy.

Key words

adalimumab, rheumatoid arthritis, methotrexate, genetic markers

Alla Skapenko, PhD
 Josef S. Smolen, MD
 Arthur Kavanaugh, MD
 Vipin Arora, PhD[§]
 Hartmut Kupper, MD
 Hendrik Schulze-Koops, MD, PhD

Please address correspondence to:

Dr Hendrik Schulze-Koops,
 Division of Rheumatology
 and Clinical Immunology,
 Med. Klinik und Poliklinik IV,
 University of Munich,
 Pettenkoferstraße 8a,
 80336 Munich, Germany.
 E-mail: hendrik.schulze-koops@med.uni-
 muenchen.de

Received on August 13, 2018; accepted in
 revised form on December 3, 2018.

© Copyright CLINICAL AND
 EXPERIMENTAL RHEUMATOLOGY 2019.

Funding: AbbVie Inc sponsored the study (ClinicalTrials.gov identifier NCT00420927); contributed to its design; and participated in the collection, analysis, and interpretation of the data, and in the writing, reviewing, and approval of the final version.

Competing interests: A. Skapenko has declared no competing interests; J. Smolen received grants for his institution from AbbVie, Amgen, AstraZeneca, Janssen, Lilly, MSD, Novartis, Pfizer, and Roche, and provided expert advice or had symposia speaking engagements for AbbVie, Amgen, Astra-Zeneca, Astro, BMS, Celgene, Celltrion, Chugai, Gilead, ILTOO, Janssen, Lilly, MSD, Novartis-Sandoz, Pfizer, Roche, Samsung, Sanofi, and UCB; A. Kavanaugh has provided expert advice to and/or received research grants from AbbVie, Amgen, AstraZeneca, BMS, Celgene, Centocor-Janssen, Pfizer, Roche, and UCB; V. Arora is a former AbbVie employee and may hold AbbVie stock; [§]current affiliation Eli Lilly and Co., Indianapolis IN, USA; H. Kupper is a full-time employee of AbbVie and may hold AbbVie stock and stock options; H. Schulze-Koops has received research grants and/or consulting fees or other remunerations from AbbVie, Abbott, Actelion, Biotest, BMS, Chugai, Essex, GSK, MSD, Medac, Merck, Mundai Pharma, Novartis, Nycomed, Pfizer, Roche, and UCB.

Introduction

Rheumatoid arthritis (RA), one of the most common systemic autoimmune diseases, affects 0.5% to 1.0% of adults in industrialised countries (1). RA is characterised by progressive joint damage, which can result in disability and decreased quality of life (1). Tumour necrosis factor (TNF) overproduction initiates synovial inflammation and joint destruction in RA (1, 2). TNF inhibitors, such as adalimumab (ADA), often in combination with methotrexate (MTX) can substantially improve treatment outcomes for RA patients (1). However, the most effective targeted treatment strategies for RA have not yet been determined for all patient populations.

The Optimal Protocol for Treatment Initiation with MTX and ADA (OPTIMA) study compared the safety and efficacy of ADA + MTX combination therapy with MTX monotherapy (*i.e.* placebo [PBO] + MTX) (3, 4). In the OPTIMA study, a greater percentage of patients receiving ADA + MTX compared with patients receiving PBO + MTX achieved stable low disease activity (LDA; 44% vs. 24%, respectively; $p < 0.001$) at week 26 (3). ADA + MTX therapy was also associated with significantly higher American College of Rheumatology (ACR) response rates, greater number of clinical remissions, absence of radiographic progression, and normal functional status compared with MTX monotherapy (3).

The genetic background of an individual may affect the manifestation, severity, and progression of RA as well as account for variability in therapeutic responses. RA is a complex multifactorial disease that develops from interrelated genetic, infectious, and environmental factors (2). One genetic region consistently associated with RA across all populations is the human leukocyte antigen locus (HLA) on chromosome 6. Specific alleles of the HLA-DRB1 gene that encode a 5-amino acid sequence motif in positions 70 to 74 of the HLA-DR β chain referred to as the shared epitope (SE) have been linked to RA susceptibility (5-7). In addition, HLA-DRB1 SE may modulate the severity of RA in affected patients (8). Autoantibodies, such as anti-citrullinated pro-

tein antibodies, are more likely to occur in patients with RA who are positive for the SE (9). The presence and titer of autoantibodies have been linked to higher disease activity and progressive joint damage (10, 11). TNF production triggered by immune complexes could account for increased disease activity and bone destruction (12). However, the role of SE in therapeutic response is not well understood.

Additional candidate loci affecting RA progression and severity include the interleukin-4 receptor (IL4R) and the low affinity immunoglobulin G (IgG) Fc gamma receptor IIb (Fc γ RIIb) variant (13-15). The rs1805010 adenine (A) to guanine (G) single nucleotide polymorphism (SNP) in the IL4R gene leads to isoleucine (I) to valine (V) amino acid substitution at position 50 of the protein sequence, referred to as I50V IL4R. It is strongly associated with early joint erosion in patients who have not yet developed bone pathology (13, 14). The rs1050501 thymine (T) to cytosine (C) SNP in the Fc γ RIIb coding sequence results in I to threonine (T) substitution at position 232, referred to as I232T Fc γ RIIb. This SNP may be a predictor of rapid radiologic joint damage in patients with definitive erosive disease (15).

Associations of genetic markers and clinical response to immunosuppressive therapy have been previously reported, but in general are derived from smaller cohorts of patients suffering from rather heterogenous disease activity, duration and prior- and/or comedication. Here, we analysed the contribution of the three candidate genetic markers to clinical and radiographic response to ADA + MTX therapy or MTX monotherapy in the OPTIMA study, taking advantage of a so far unbeknown rather large patient cohort of 894 individuals with homogenous, clinically well-defined, early RA undergoing randomised, standardised treatment with controlled and pre-defined clinical outcome measures.

Methods

Study design and patient population

Detailed OPTIMA study design and patient disposition are presented in Kavanaugh *et al.* (3). Briefly, OPTIMA was a phase 4, double-period, double-

blind, randomised, multicentre, controlled trial. Adults aged ≥ 18 years with RA diagnosis (< 1 year duration) based on 1987-revised ACR classification criteria, and no prior exposure to MTX or anti-TNF treatment were eligible for this study. Patients must also have met the following criteria: 1. disease activity score based on 28 joints and C-reactive protein (DAS28-CRP) > 3.2 ; 2. tender joint count based on 68 joints (TJC68) of ≥ 8 ; 3. swollen joint count based on 66 joints (SJC66) of ≥ 6 ; 4. erythrocyte sedimentation rate ≥ 28 mm/h or CRP ≥ 1.5 mg/dL; 5. rheumatoid factor positive (RF+), anti-cyclic citrullinated peptide positive (anti-CCP+), or > 1 erosion at the joints of hands or feet by x-ray (3). Patients provided separate written informed consent to participate in the genetic analysis, which was approved by the Hospital of the University of Munich ethics committee. Data from period 1, in which the patients were assigned 1:1 to receive ADA 40 mg subcutaneously every other week + MTX titrated to 20 mg/week orally by week 8 or matching PBO + MTX doses for 26 weeks (Fig. 1) (3), were included in this analysis.

Clinical and radiographic assessments
Clinical efficacy endpoints for this genetic analysis were the percentage of patients achieving LDA (DAS28-CRP < 3.2), remission (DAS28-CRP < 2.6), and ACR 20%, 50% and 70% response criteria (ACR50) at week 26.

Radiographic endpoints included change from baseline to week 26 in modified total Sharp score (Δ mTSS) > 0.5 and the percentage of patients with rapid radiographic progression (RRP; Δ mTSS > 1.5). Additional endpoints included mean Δ mTSS, joint erosion (JE), and joint space narrowing (JSN). Two blinded readers scored radiographs of 23 bilateral joints of the hand/wrist and forefoot for JE (scored 0 to 5 per joint; range, 0 to 230) and scored 21 bilateral joints of the hand/wrist and forefoot for JSN (scored 0 to 4 per joint; range, 0 to 168).

Genetic analysis

At baseline, whole peripheral blood samples were obtained from OPTIMA

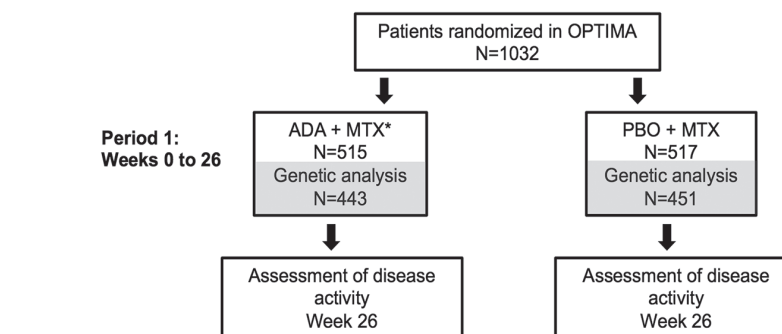


Fig. 1. OPTIMA study design and patient disposition for period 1.

ADA: adalimumab; DAS28(CRP): Disease Activity Score using a 28-joint count and C-reactive protein level; LDA: low disease activity; MTX: methotrexate; PBO: placebo. *MTX titrated to 20 mg/wk by week 8.

study patients, shipped and kept frozen at the central laboratory until DNA isolation. Genomic DNA was isolated from white blood cells using the Qiagen® Midikit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The number of HLA-DRB1 SE copies (0, 1, or 2) was determined using high resolution sequencing (Protrans, Hockenheim, Germany). The I50V IL4R (rs1805010) and I232T FcγRIIb (rs1050501) SNPs were genotyped by allele-specific Assay-on-Demand PCR (Applied Biosystems, Carlsbad, CA). To control for potential bias when associating the clinical and the genetic data, the genetic data of the individuals were not made available to the team evaluating the clinical response of the patients and the clinical data of individual patients were not known to the research team performing the genetic analyses.

Statistical methods

Non-responder imputation was used for LDA and clinical efficacy outcomes, with missing responses considered as non-responders. Cochran-Mantel-Haenszel test was used to evaluate differences within treatment groups and chi-square test or Fisher's exact test was used to evaluate allele distribution among treatment groups. The effect of individual alleles as well as interaction between treatment and alleles on clinical response were estimated using logistic regression, after adjustment for baseline characteristics (anti-CCP+, CRP level, DAS28, presence of erosions, RF+, SJC66, TJC68, sex, and smoking status). The chi-square test, univariate and multivariate regression,

and Pearson product-moment correlation were used to determine the relationship between number of variant alleles and percentage of patients demonstrating radiographic progression, RRP, Δ mTSS, JE, and JSN. Safety parameters were summarised by genetic variant in each treatment arm. For all statistical comparisons, Bonferroni corrections were performed to address multiple comparisons and the risk of false positive findings, and a 2-sided $p < 0.05$ was considered statistically significant (Supplementary Table I).

Results

Patient disposition and allele distribution

In the OPTIMA study, 1032 patients were randomised to either ADA + MTX ($n=515$) or PBO + MTX ($n=517$) in period 1 (3). For this analysis, 894 participants (87%) provided genetic information, 443 and 451 patients in the ADA + MTX and PBO + MTX arms, respectively (Fig. 1 and Table I). Radiographic and genetic information were available for 829 patients in this subanalysis. Baseline demographic and disease characteristics of patients were similar in both treatment arms for this genetic analysis (Table I).

HLA-DRB1 SE copy numbers and the I50V IL4R variants were distributed similarly in each treatment group; however, the I232T FcγRIIb variants were unequally distributed ($p=0.03$; Supplementary Table II).

Among the I50V IL4R and I232T FcγRIIb variants, no significant differences in racial distribution were observed between treatment groups

Table I. Demographic and clinical characteristics of patients with RA in the OPTIMA genetic analysis at baseline.

Characteristic	ADA + MTX (n=443)	PBO + MTX (n=451)
Male to Female, %	28:72	26:74
Age, mean ± SD, y	50.9 ± 13.9	50.4 ± 13.6
White race, n (%)	395 (89)	406 (90)
Current smoker, n (%)	109 (25)	111 (25)
RF+ status, n/N (%)	376/438 (86)	397/447 (89)
Anti-CCP+ status, n/N (%)	364/438 (83)	372/448 (83)
RF+ and anti-CCP+, n/N (%)	341/434 (79)	355/446 (80)
TJC68 count, mean ± SD	29.4 ± 15.0	27.7 ± 14.6
SJC66 count, mean ± SD	18.6 ± 10.6	18.0 ± 10.4
CRP, mean ± SD, mg/dL	27.2 ± 30.1	30.8 ± 33.6
DAS28(CRP) score, mean ± SD	6.1 ± 0.95	6.0 ± 0.98
HAQ index, mean ± SD	1.6 ± 0.7	1.6 ± 0.7

ADA: adalimumab; CCP: cyclic citrullinated peptide; CRP: C-reactive protein; DAS28(CRP): disease activity score using a 28-joint count and CRP level; HAQ: Health Assessment Questionnaire; MTX: methotrexate; PBO: placebo; RA: rheumatoid arthritis; RF: rheumatoid factor; SD: standard deviation; SJC66: swollen joint count based on 66 joints; TJC68: tender joint count based on 68 joints.

Table II. Differences in baseline mean (SD) mTSS between ADA+MTX and PBO+MTX treatment groups based on I50V IL4R genotype.

I50V IL4R Genotype	ADA + MTX (n=438)	PBO + MTX (n=449)	Total (n=887)	p-value*
AA (I50/I50)	12.2 (16.2) n=143	11.7 (20.2) n=130	12.0 (18.2) n=273	0.819
AG (I50/V50)	11.0 (18.0) n=208	11.6 (18.0) n=232	11.3 (18.0) n=440	0.749
GG (V50/V50)	11.8 (19.8) n=87	9.8 (15.1) n=87	10.8 (17.6) n=174	0.452

All values are mean (SD).

*p-values from one way analysis of variance.

ADA: adalimumab; IL4R: interleukin-4 receptor; MTX: methotrexate; PBO: placebo.

Table III. Differences in baseline mean (SD) DAS28, TJC, SJC, and CRP levels between ADA+MTX and PBO+MTX groups categorised by HLA-DRB1 SE copy variants.

HLA-DRB1 SE Genotype	ADA + MTX	PBO + MTX	Total	p-value*
0 copies	n=145	n=167	n=312	
DAS28 [†]	6.0 (1.0)	6.1 (1.0)	6.1 (1.0)	0.616
TJC	31.0 (15.2)	29.3 (14.8)	30.1 (15.0)	0.337
SJC	19.2 (11.6)	19.1 (11.2)	19.1 (11.4)	0.912
CRP	25.2 (19.1)	28.7 (34.0)	27.1 (31.9)	0.336
1 copy	n=216	n=215	n=431	
DAS28 [‡]	6.0 (1.0)	6.0 (1.0)	6.0 (1.0)	0.809
TJC	29.1 (15.2)	26.5 (14.2)	27.8 (14.8)	0.073
SJC	19.0 (10.7)	17.8 (10.4)	18.4 (10.6)	0.232
CRP	27.6 (31.9)	32.3 (33.0)	29.9 (32.5)	0.127
2 copies	n=82	n=69	n=151	
DAS28 [§]	6.1 (0.8)	5.9 (0.9)	6.0 (0.9)	0.137
TJC	27.8 (14.2)	27.4 (14.9)	27.6 (14.5)	0.865
SJC	16.5 (8.0)	16.1 (8.1)	16.3 (8.0)	0.781
CRP	29.8 (26.8)	31.0 (34.6)	30.4 (30.5)	0.813

All values are mean (SD).

*p-values from one way analysis of variance. [†]ADA+MTX, n=141; PBO+MTX, n=160; total, n=301.

[‡]ADA+MTX, n=214; PBO+MTX, n=214; total, n=428. [§]ADA+MTX, n=80; total, n=149.

ADA: adalimumab; HLA-DRB1 SE: human leukocyte antigen DRB1 SE; IL4R: interleukin-4 receptor; MTX: methotrexate; PBO: placebo.

(Supplementary Table III). Similarly, among the HLA-DRB1 SE copy variants, there were no significant differences in racial distribution between the treatment groups except for those with 2 copies of HLA-DRB1 SE (Supplementary Table II). The baseline mean mTSS was similar between treatment groups among the I50V IL4R genotype variants (Table II). Similarly, no significant differences between treatment groups were observed in mean DAS28, TJC, SJC, and CRP levels among the HLA-DRB1 SE copy variants at baseline (Table III).

Genetic associations with clinical response at week 26

With increasing HLA-DRB1 SE copy number, the frequency of patients achieving LDA ($p=0.02$), ACR20 ($p=0.02$), ACR50 ($p=0.01$), ACR70 ($p=0.01$) and DAS28 remission ($p=0.30$) increased in the ADA + MTX group, while in the PBO + MTX group the response rate numerically decreased with increasing SE copy number (Fig. 2A). In the ADA + MTX group, SE copy number (2 copies vs. 0 copies) was significantly associated with achieving a status of clinically meaningful low disease activity, such as LDA (odds ratio [OR], 2.08; 95% confidence interval [CI], 1.12–3.88; $p=0.02$) or ACR50 (OR, 2.00; CI, 1.07–3.72; $p=0.03$). In the PBO + MTX arm, there was a negative trend for SE copy numbers and LDA for 1 copy versus 0 copies (OR, 0.58; CI, 0.35–0.98; $p=0.04$) and for ACR50 for 1 and 2 copies versus 0 copies (OR, 0.58; CI, 0.36–0.92; $p=0.02$ and OR, 0.46; CI, 0.24–0.90; $p=0.02$, respectively).

Patients with IL4R GG genotype (V50/V50) showed a decrease in LDA responses to ADA + MTX and an increase with PBO + MTX treatment (Fig. 2B), but these comparisons were not statistically significant. IL4R SNP was not associated with DAS28 remission or ACR50 in either treatment group. Patients with FcγRIIb CC genotype (T232/T232) in the ADA + MTX group showed a non-significant marginal increase in LDA and ACR50 responses. FcγRIIb CC genotype was significantly associated with DAS28 remission in an anal-

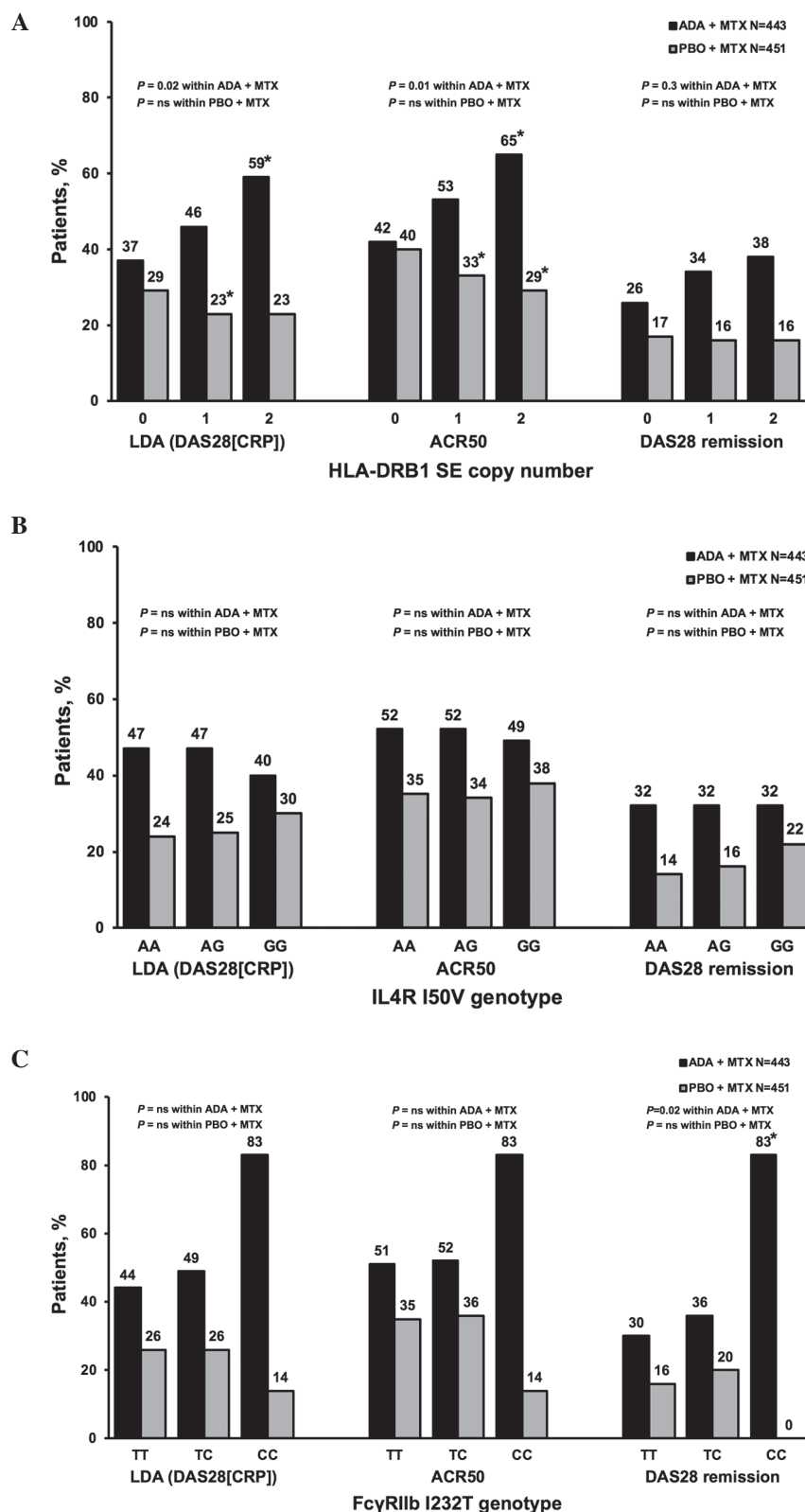


Fig. 2. Percentage of patients achieving LDA, ACR50, and DAS28 remission at week 26 by (A) SE copy number, (B) I50V IL4R genotype, and (C) I232T Fc_γRIIb genotype. *p*-values for within treatment comparison calculated from Cochran-Mantel-Haenszel test. *p*-values for pairwise comparison calculated from chi-square or Fisher's exact test. ***p*<0.01; **p*<0.05. ACR50: American College of Rheumatology 50% response criteria; ns: non significant; ADA: adalimumab; DAS28: Disease Activity Score using a 28-joint count; DAS28(CRP): DAS28 and C-reactive protein level; Fc_γRIIb: Fc gamma receptor IIb; HLA-DRB1 SE: human leukocyte antigen DRB1 SE; IL4R: interleukin-4 receptor; LDA: low disease activity; MTX: methotrexate; PBO: placebo; SE: shared epitope.

ysis (*p*<0.02; Fig. 2C) using a logistic regression model. Fc_γRIIb CC genotype was a significant predictor of remission in the ADA + MTX group (OR, 17.49; CI, 1.80–170.39; *p*=0.01) compared with the TT genotype (1232/1232).

Genetic associations with radiographic endpoints

In this study, patients treated with ADA + MTX experienced less radiographic progression from baseline to week 26 than the PBO + MTX group. There were no significant correlations between SE copy number or number of Fc_γRIIb C alleles related to radiographic progression (Δ*m*TSS >0.5) or RRP (Δ*m*TSS >1.5) in either treatment group (Fig. 3A-B). The IL4R genotype did not correlate with radiographic progression in the ADA + MTX group (Fig. 3A). However, the numbers of IL4R G allele was significantly associated with radiographic progression (Pearson *r*=0.99; *p*=0.04) and RRP in the PBO + MTX arm (Pearson *r*=1.0; *p*=0.03) at week 26 (Fig. 3A-B). These results replicate previous findings that identified IL4R I50V as a marker of early joint erosion (13). No significant genetic effects were observed for JE or JSN.

Genetic associations with safety parameters

The most common AEs (frequency >5%) in the OPTIMA study at week 26 were nausea, upper respiratory tract infection, and nasopharyngitis in the ADA + MTX group and nausea, upper respiratory tract infection, and diarrhea in the PBO + MTX group (3). While frequencies of AEs were similar between most genetic variants in each treatment group, there was a significant relationship between I50V IL4R variants and common AEs in the ADA + MTX group. Percentage of patients with the IL4R GG genotype who experienced these AEs was 40.9% compared with 28.6% and 26.2% for AG and AA genotypes, respectively (chi-square *p*=0.02).

Discussion

Treatment with ADA + MTX in the OPTIMA study produced significantly higher DAS28 LDA, ACR20, ACR50, ACR70 and DAS28 remission respon-

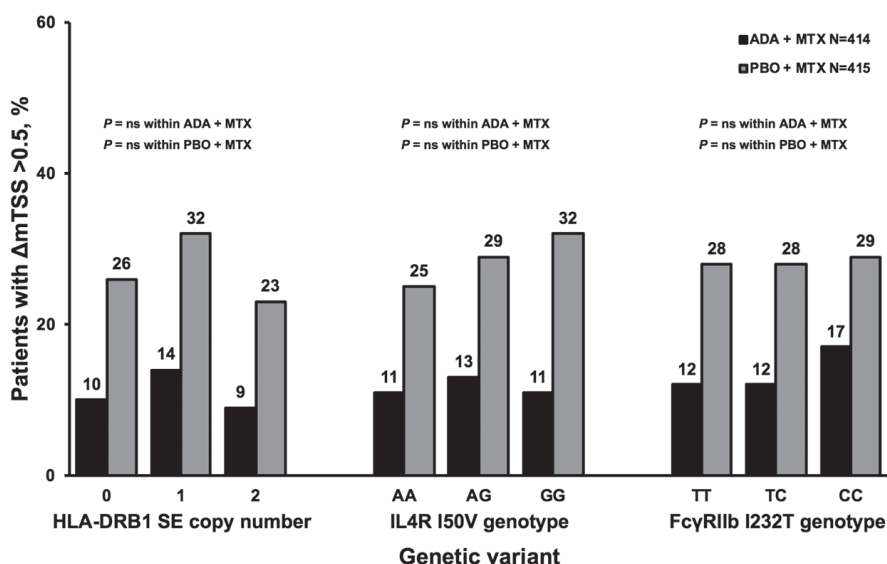


Fig. 3. Percentage of patients with (A) radiographic progression ($\Delta mTSS > 0.5$) and (B) RRP ($\Delta mTSS > 1.5$) at week 26 by SE copy number and I50V IL4R and I232T Fc γ RIIb genotypes. *p*-values from Pearson correlation. Correlation between Fc γ RIIb TT genotype and RRP was not calculated within ADA + MTX owing to small numbers of patients. ns: non significant; ADA: adalimumab; Fc γ RIIb: Fc gamma receptor IIb; HLA-DRB1 SE: human leukocyte antigen DRB1 SE; IL4R: interleukin-4 receptor; mTSS: modified total Sharp score; MTX: methotrexate; PBO: placebo; RRP: rapid radiographic progression; SE: shared epitope.

ses at week 26 compared with PBO + MTX, regardless of genetic background, in patients with early RA (3). In this genetic analysis, HLA-DRB1 SE copy number was significantly associated with improvements in LDA and ACR20, ACR50 and ACR70 responses in patients treated with ADA + MTX, but not PBO + MTX at week 26. Fc γ RIIb T232/T232 variant predicted remission in response to ADA + MTX treatment, but these results should be interpreted with caution due to smaller sample size. The I50V IL4R variant was confirmed as a marker of radiographic progression in early RA, potentially interacting with the SE to enhance clinical response.

Overall, this post hoc analysis of a homogenous cohort of patients with early RA from a large, randomised-controlled study is unique in several aspects. This study population derives from a randomised controlled clinical trial and is therefore rather homogeneous with regard to disease duration, disease activity, current and previous medication, treatment while under investigation and predefined clinical outcome measures. The data validate the results of previous observations obtained from heterogeneous (and small) patient groups and are

instrumental for the field as they confirm that some genetic markers are associated with the severity of RA as well as response to treatment.

The estimates of genetic risk contributed by HLA locus in RA susceptibility range from 11% (16) to 50% (5, 13). The broad observed range could be due to the presence of both risk and protective alleles within this region. Viatte *et al.* have reported association of SE with both anti-CCP+ (seropositive) and anti-CCP- (seronegative) RA, but the latter group had smaller effect size (effect size ratio = 3.2) (17). The mechanistic role of SE in RA susceptibility is still under investigation. The genetic risk for seropositive RA is contributed by 5 specific amino acids (2 within the SE) located in the HLA antigen-binding groove, potentially altering binding affinity (18, 19). The SE plays a role in Th17 cell polarisation, which contributes to the pathogenesis of autoimmune diseases such as RA (6, 7). Patients with SE exhibit more severe radiographic progression, supporting the contribution of this epitope to disease progression and severity (7, 8, 20). Still, this analysis also demonstrates that SE is associated with improved responses to anti-TNF + MTX therapy in early RA

patients. Increasing efficacy of ADA + MTX treatment with SE copy number may be indicative of regulation of TNF levels by SE (12). Interestingly, LDA and ACR20, ACR50 and ACR70 responses significantly increased with increasing SE copy number in the ADA + MTX treatment group but showed a decreased trend with increasing SE copy number in the MTX + PBO group, suggesting that this association may represent a pharmacogenetic response.

The genetic associations found in this study is corroborated by earlier findings identifying I50V IL4R and I232T Fc γ RIIb SNPs as markers of erosive disease and joint damage in RA (13, 15). Increased frequency of bone erosions occurred within 2 years of RA diagnosis in patients carrying I50V/I50V IL4R variant along with HLA-DRB1 SE and RF+ markers (13, 14). These results may be explained by reduced responsiveness of I50V receptor variant to IL4 ligand. Importantly, the data provided here also show for the first time that early treatment with a TNF inhibitor prevents bone erosion even in patients with two IL4R risk alleles. This finding may suggest that genetic stratification at baseline would be beneficial to prevent bone erosions in high-risk patients. In patients with established erosive RA, the Fc γ RIIb I232T variant was associated with increased radiographic progression during the first 6 years. Dendritic cells from these patients displayed reductions in immune complex uptake and alterations in cytokine profiles, which may be related to severity of RA (15).

Only a few genetic studies on RA susceptibility, including large genome-wide association studies, have been replicated (21-23). A recent genome-wide study evaluated changes in disease activity score after 14 weeks of anti-TNF therapy and identified 8 genetic loci accounting for 3.8% of the variance in treatment response (24). However, no single genetic variant reached genome-wide significance, possibly due to relatively small sample size (2703 RA patients receiving anti-TNF treatment), disease heterogeneity, or differences in anti-TNF agents and additional medication use (24). In a study evaluating

association of TNF and SE genotypes with clinical response to ADA and ADA + MTX in patients from the ReAct trial, a distinct haplotype of the TNF gene (-238G, -308G, -857C) was associated with a reduced ACR50 response rate at week 12. However, no such association was detected for the SE carrier status or copy number. These contradicting results may be attributed to relatively small genetic analysis sample size in the ReAct study (388 vs. 829 patients in the ReAct and OPTIMA trials, respectively); nature of the study cohort (patients with long standing disease and intense pre-treatment *versus* patients with early RA and absence of biological pre-treatment); and the shorter time of evaluation (12 weeks in the ReAct trial vs. 26 weeks in the OPTIMA study) (25). However, in an early RA study that compared MTX treatment with TNF inhibitor etanercept, the presence of two HLA-DRB1 SE alleles was significantly associated with response to etanercept treatment as measured by ACR50 response at 12 months (OR: 4.3; 95% CI: 1.8–10.3) (26). Our results are in line with this and provide evidence that the HLA-DRB1 SE contributes to treatment response to anti-TNF therapy in early RA and that the I50V IL4R variant is associated with early erosive RA disease.

The three genetic markers evaluated in our analysis were chosen for several reasons: the HLA-DRB1 SE is a commonly investigated gene in RA and has robust data on association and outcome, the IL4R and FcγIIIR are associated with erosive disease, and all three genes have been associated with RA and bone erosions in previous studies. Limitations of this genetic analysis include relatively modest sample size, lack of statistical power, and lack of primary and confirmatory groups. In addition, despite corrections for multiple comparisons, the risk for type 1 errors in the analysis cannot completely be excluded. Although a significant association between HLA-DRB1 SE copy number and treatment response was demonstrated in the current analysis, given the prevalence of the SE in RA, some association with treatment response, lack of response, or toxic-

ity is not unexpected. In this analysis, statistically significant association between I50V IL4R variant and radiographic progression was detected. Yet, relatively short follow-up time and low overall radiographic progression may have masked the stronger correlation observed in previous studies. Similar frequencies of AEs were observed across different genetic variants except association of I50V IL4R variant with nausea, upper respiratory tract infection, and nasopharyngitis in the ADA + MTX group. In the OPTIMA study, frequencies of AEs were comparable between treatment arms through week 26 (3). However, a greater number of serious infections and deaths occurred in the ADA + MTX group, possibly due to increased age and comorbidities (3). Early effective therapeutic intervention in RA has consistently prevented long-term joint damage and disability. Hence, there is a need to identify patients with RA who have an increased risk of developing an aggressive disease course and those who may benefit from specific treatment options (14, 28). Precise targeting of anti-TNF agents based on genetic biomarkers has the potential to improve clinical responses and quality of life for patients as well as reduce healthcare costs (17, 29).

Conclusion

These analyses suggest that certain genetic variants have a stronger influence on initial clinical response to anti-TNF agents in patients with early RA rather than on sustained disease control. Identification and understanding of critical genetic components that contribute to clinical and radiographic response to TNF antagonists may help identify patients at risk of aggressive disease progression and consequently guide treatment decisions in patients with early RA.

Acknowledgments

Editorial assistance was provided under the direction of the authors by Rebecca Slager, PhD, MS, and Jennifer Rossi at MedThink SciCom and Maria Hovenden, PhD, of Complete Publication Solutions, LLC (North Wales, PA) and was funded by AbbVie.

References

- SCOTT DL, WOLFE F, HUIZINGA TW: Rheumatoid arthritis. *Lancet* 2010; 376: 1094-108.
- CALABRESI E, PETRELLI F, BONIFACIO AF, PUXEDDU I, ALUNNO A: One year in review 2018: pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol* 2018; 36: 175-84.
- KAVANAUGH A, FLEISCHMANN RM, EMERY P *et al.*: Clinical, functional and radiographic consequences of achieving stable low disease activity and remission with adalimumab plus methotrexate or methotrexate alone in early rheumatoid arthritis: 26-week results from the randomised, controlled OPTIMA study. *Ann Rheum Dis* 2013; 72: 64-71.
- SMOLEN JS, EMERY P, FLEISCHMANN R *et al.*: Adjustment of therapy in rheumatoid arthritis on the basis of achievement of stable low disease activity with adalimumab plus methotrexate or methotrexate alone: the randomised controlled OPTIMA trial. *Lancet* 2014; 383: 321-32.
- BOWES J, BARTONA A: Recent advances in the genetics of RA susceptibility. *Rheumatology* 2008; 47: 399-402.
- DE ALMEIDA DE, LING S, HOLOSHITZ J: New insights into the functional role of the rheumatoid arthritis shared epitope. *FEBS letters* 2011; 585: 3619-26.
- DE ALMEIDA DE, LING S, PI X, HARTMANN-SCRUGGS AM, PUMPENS P, HOLOSHITZ J: Immune dysregulation by the rheumatoid arthritis shared epitope. *J Immunol* 2010; 185: 1927-34.
- CALIN A, ELSWOOD J, KLOUDA PT: Destructive arthritis, rheumatoid factor, and HLA-DR4. Susceptibility versus severity, a case-control study. *Arthritis Rheum* 1989; 32: 1221-5.
- HOLOSHITZ J: The rheumatoid arthritis HLA-DRB1 shared epitope. *Curr Opin Rheumatol* 2010; 22: 293-8.
- FARHEEN K, AGARWAL SK: Assessment of disease activity and treatment outcomes in rheumatoid arthritis. *J Manag Care Pharm* 2011; 17: S9-13.
- HÄUPL T, SKAPENKO A, HOPPE B *et al.*: [Biomarkers and imaging for diagnosis and stratification of rheumatoid arthritis and spondylarthritis in the BMBF consortium ArthroMark]. *Z Rheumatol* 2018; 77 (Suppl. 1): 16-23.
- BRAUN T, ZWERINA J: Positive regulators of osteoclastogenesis and bone resorption in rheumatoid arthritis. *Arthritis Res Ther* 2011; 13: 235.
- PROTS I, SKAPENKO A, WENDLER J *et al.*: Association of the IL4R single-nucleotide polymorphism I50V with rapidly erosive rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 1491-500.
- SKAPENKO A, PROTS I, SCHULZE-KOOPS H: Prognostic factors in rheumatoid arthritis in the era of biologic agents. *Nat Rev Rheumatol* 2009; 5: 491-6N
- RADSTAKE TR, FRANKE B, WENINK MH *et al.*: The functional variant of the inhibitory Fcγ receptor IIb (CD32B) is associated with the rate of radiologic joint damage and dendritic cell function in rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 3828-37.
- KURKÓ J, BESENYEI T, LAKI J, GLANT TT,

- MIKECZ K, SZEKANECZ Z: Genetics of rheumatoid arthritis - a comprehensive review. *Clin Rev Allergy Immunol* 2013; 45: 170-79.
17. VIATTE S, PLANT D, BOWES J *et al.*: Genetic markers of rheumatoid arthritis susceptibility in anti-citrullinated peptide antibody negative patients. *Ann Rheum Dis* 2012; 71: 1984-90.
 18. VAN VOLLENHOVEN RF: Rheumatoid arthritis in 2012: progress in RA genetics, pathology and therapy. *Nat Rev Rheumatol* 2013; 9: 70-2.
 19. RAYCHAUDHURI S, SANDOR C, STAHL EA *et al.*: Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat Genet* 2012; 44: 291-6.
 20. MORENO I, VALENZUELA A, GARCÍA A, YÉLAMOS J, SÁNCHEZ B, HERNÁNZ W: Association of the shared epitope with radiological severity of rheumatoid arthritis. *J Rheumatol* 1996; 23: 6-9.
 21. RAYCHAUDHURI S, REMMERS EF, LEE AT *et al.*: Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet* 2008; 40: 1216-23.
 22. RAYCHAUDHURI S, THOMSON BP, REMMERS EF *et al.*: Genetic variants at CD28, PRDM1 and CD2/CD58 are associated with rheumatoid arthritis risk. *Nat Genet* 2009; 41: 1313-8.
 23. PRAJAPATI R, PLANT D, BARTON A: Genetic and genomic predictors of anti-TNF response. *Pharmacogenomics* 2011; 12: 1571-85.
 24. UMIČEVIĆ MIRKOV M, CUI J, VERMEULEN SH *et al.*: Genome-wide association analysis of anti-TNF drug response in patients with rheumatoid arthritis. *Ann Rheum Dis* 2013; 72: 1375-81.
 25. MICELI-RICHARD C, COMETS E, VERSTUYFT C *et al.*: A single tumour necrosis factor haplotype influences the response to adalimumab in rheumatoid arthritis. *Ann Rheum Dis* 2008; 67: 478-84.
 26. CRISWELL LA, LUM RF, TURNER KN *et al.*: The influence of genetic variation in the HLA-DRB1 and LTA-TNF regions on the response to treatment of early rheumatoid arthritis with methotrexate or etanercept. *Arthritis Rheum* 2004; 50: 2750-6.
 27. MEWAR D, WILSON AG: Treatment of rheumatoid arthritis with tumour necrosis factor inhibitors. *Br J Pharmacol* 2011; 162: 785-91.
 28. IBRAHIM I, OWEN SA, BARTON A: Genetics and the impact on treatment protocols in patients with rheumatoid arthritis. *Expert Rev Clin Immunol* 2012; 8: 509-11.
 29. BAHLAS S, DAMIATI L, DANDACHI N, SAIT H, ALSEFRI M, PUSHPARAJ PN: Rapid immunoprofiling of cytokines, chemokines and growth factors in patients with active rheumatoid arthritis using Luminex Multiple Analyte Profiling technology for precision medicine. *Clin Exp Rheumatol* 2019; 37: 112-19.