Retrospective Genetic Analysis of Efficacy and Adverse Events in a Rheumatoid Arthritis Population Treated with Methotrexate and Anti-TNF-α

Foti A[1], Lichter D[1], Shadick NA[2], Maher NE[2], Ginsburg GS[3], Lekstrom-Himes J[1], Meyer J[4], Weinblatt ME[2], Parker A[1]
[1]Millennium Pharmaceuticals, Cambridge MA; [2]Brigham and Women's Hospital, Boston MA; [3]Duke University, Durham, NC; [4]Novartis AG, Cambridge MA

ABSTRACT

Background:
Progress has been made in the treatment of rheumatoid arthritis (RA) but there remain a large number of patients who do not respond to therapy and/or experience drug-related adverse events (AEs).

Objective:
To identify genetic markers associated with efficacy and predisposition to adverse events during methotrexate (MTX) therapy or TNF-a blockade.

Methods:
The study cohort was selected from a large RA patient registry. Non-responders were patients who discontinued therapy due to no efficacy after 3 to 18 months (MTX; N=21) or 1 to 18 months (anti-TNF-a; N=17). Controls were defined as currently treated patients who have been on therapy for at least 3 months (MTX; N=104, all anti-TNF-a naive) or 1 month (anti-TNF-a; N=124). AE cases were patients who reported discontinuing therapy due to any AE (MTX, N = 64; anti-TNF-a, N = 19). Among these, we defined a subgroup of severe AEs (liver or pulmonary toxicity, anemia, neutropenia, and infections; MTX, N = 29; anti-TNF-a, N = 7), in contrast to milder AEs such as headaches and alopecia; controls were patients who are currently receiving therapy without reported AEs (MTX, N=180, mean exposure 58 months, SD = 64; anti-TNF-a, N=132, mean exposure 25 months, SD = 20).
31 genes were selected for analysis on the basis that they were found to associate with RA risk or symptom severity in at least two published studies. We genotyped 60 SNPs, 9 VNTRs and the HLA-DRB1 locus.

Results:
Using contingency table analyses five loci were associated with lack of efficacy in MTX therapy (CTLA4, p = 0.033; IL1B, p = 0.007; TNF, p = 0.021; RUNX1, p = 0.003; SLC11A1, p=0.008), and three loci with lack of efficacy of TNF-a blockade (FcGR2A, p = 0.018; IL1RN, p = 0.009; IL4R, p = 0.046). Only one locus was associated with MTX AEs (IL1B, p = 0.014), and four with AEs during TNF-a blockade (HLA-DRB1, p = 0.037; IFNG, p = 0.049; IL3 p = 0.025, SLC19A1, p = 0.043). However, when the analysis was restricted to severe adverse events the number of associated loci increased to two for MTX therapy (HLA-DRB1, p = 0.033; CCR5, p = 0.007), and six for anti-TNF-a therapy (IL3, p = 0.007; TNF, p = 0.015; IL4R, p = 0.023; PADI4, p = 0.02; SLC19A1, p = 0.03; SLC22A4, p = 0.050). Multivariate logistic regression yielded nominally significant results for MTX efficacy (RR = 6.38, p<0.0001), MTX severe ADR (RR = 8.01, p = 0.01) and anti-TNF-a severe ADR (RR = 21.7, p = 0.05).

Conclusions:
Together, these results indicate a significant genetic component to the efficacy and toxicological profiles of two common RA therapies. The non-overlapping sets of efficacy-associated genes suggest the potential for therapy-specific markers, but our results also imply a central role for cytokines and their receptors in RA pharmacogenetics.

INTRODUCTION

While great advances have been made in the treatment of rheumatoid arthritis (RA), even newer therapies such as TNF-alpha blockade present a number of failures owing to either lack of efficacy or drug-related adverse events (AEs). For instance, a recent study of the anti-TNF-alpha agent Infliximab showed a 25% dropoff in use after 2 years (Stem and Wolfe 2004). This implies that there are a large number of patients for whom different or earlier and more aggressive therapy might prove beneficial.
Additionally, because of the slowly-progressing nature of the disease, clinical trials in RA typically last several months, resulting in substantial costs for evaluation of new therapeutic agents. Genetic biomarkers could be used to stratify or enrich clinical trial populations, as covariates for analysis of therapeutic outcome data, or as covariates in the analysis of dynamic biomarkers. Any of these uses could result in more efficient clinical trials and consequent cost-savings.
However, while the literature presents many examples of association between gene polymorphisms and severity of disease, very little is known about genetic markers of efficacy or AEs. To address this question, we selected 31 genetic loci, including HLA-DRB1, which had been implicated in either risk for or severity of RA in at least two published studies. A series of genetic markers, both VNTRs and SNPs, was then selected to characterize these genes in a recently-recruited RA registry. These were analyzed using contingency tables and multivariate logistic regression techniques.

SUBJECTS AND METHODS

RA registry patients were recruited at a major metropolitan rheumatology clinic and phenotyped using ACR diagnostic criteria, among others. All studies were carried out using IRB-approved informed consent, questionnaire, and biological sampling protocols. All individuals studied here self-described as being of European Caucasian descent; sample size and demographics are given in Table 1. Medication history, including current therapeutic regimen, was collected using a standardized self-report questionnaire. Non-responders were defined as patients who discontinued therapy due to no efficacy after 3 to 18 months (MTX; N=21) or 1 to 18 months (anti-TNF-a; N=17). Controls were defined as currently treated patients who have been on therapy for at least 3 months (MTX; N=104, all anti-TNF-a naive) or 1 month (anti-TNF-a; N=124). AE cases were patients who reported discontinuing therapy due to any AE (MTX, N = 64; anti-TNF-a, N = 19). Among these, we defined a subgroup of severe AEs (liver or pulmonary toxicity, anemia, neutropenia, and infections; MTX, N = 29; anti-TNF-a, N = 7), in contrast to milder AEs such as headaches and alopecia; controls were patients who are currently receiving therapy without reported AEs (MTX, N=180, mean exposure 58 months, SD = 64; anti-TNF-a, N=132, mean exposure 25 months, SD = 20). An overview of the reasons given for discontinuing therapy across the entire patient cohort is shown in Figure 1.

Table 1: Sample Size and Demographic Features

	Cases
Sample Size	346
Catchment Area	Boston, MA
Mean Age (Range)	58 (22-88)
Percent Female	84%
Osteoarthritis	108
Smoking (Ever)	154

Thirty-one genes were selected for analysis on the basis that they were found to associate with RA risk or symptom severity in at least two published studies. We genotyped 60 SNPs, 9 VNTRs and the HLA-DRB1 locus. Microsatellite (VNTR) genotyping was carried out using fluorescently-labeled PCR primers and standard capillary electrophoresis protocols (AB 3100). SNP genotyping was performed at Genaissance Pharmaceuticals (New Haven, CT) using single-base extension and the MassArray™ detection platform (Sequenom). HLA genotyping was conducted using AS-PCR methods based on those of Kotsch *et al.* (1999), followed by DNA sequencing where required to resolve SE and D-70 copy number.
All VNTRs were collapsed to two-allele markers following published reports of allele-specific association. Significance of single-marker associations with lack of efficacy or AEs was assessed using Fisher's exact test. All markers were evaluated assuming dominance; for those markers with minor allele frequency greater than 10% a recessive model was also tested. Single-marker associations with lack of efficacy or adverse events were evaluated using contingency table analysis. All markers that exhibited nominally significant evidence for association were included in construction of multimarker models; these used multivariate logistic regression.

Figure 1: Reasons for Discontinuation of Therapy

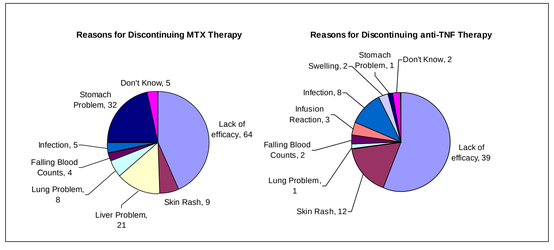
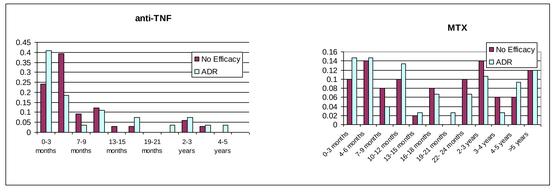


Figure 2: Length of Exposure Prior to Discontinuation of Therapy



RESULTS

We found evidence for association of five loci with lack of efficacy of MTX therapy, and three loci with lack of efficacy of TNF-a blockade (Table 2). Within the MTX group, two loci (IL1B and RUNX1) show particularly strong association (p=0.007 and p=0.003 respectively), as does IL1RN in the TNF-a blockade group (p=0.009). However, there was no statistically significant association between the Shared Epitope (SE) and response to therapy (Table 3).
We saw much weaker evidence for genetic associations with adverse events within either therapeutic group, with no p-value falling below the 0.01 threshold. However, when we included only severe adverse events (liver or pulmonary toxicity, anemia, neutropenia and infections), the number of associated loci increased from one to two in the MTX therapy group, with one locus (CCR5) reaching a p-value of 0.007, and from four to six in the TNF-a blockade group, with the most notable being IL3 (p= 0.007).
Multivariate logistic regression analyses yielded nominally significant results for prediction of MTX efficacy (RR = 6.38, p<0.0001), MTX severe AE (RR = 8.01, p = 0.01) and anti-TNF-a severe AE (RR = 21.7, p = 0.05).

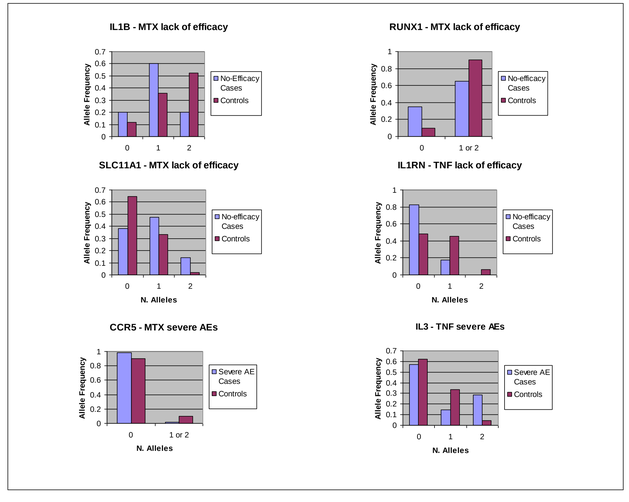
Table 2: Summary of Results

Phenotype Cohort	Drug Regimen	Locus	P-value
Lack of efficacy	MTX	CTLA4	0.0334
		IL1B	0.0079
		TNF	0.0217
		RUNX1	0.0034
		SLC11A1	0.0084
	TNF	FcGR2A	0.0176
		IL1RN	0.0086
		IL4R	0.0456
Adverse Events	MTX	IL1B	0.0140
	TNF	HLA-DRB1	0.0373
		IFNG	0.0495
		IL3	0.0405
		SLC19A1	0.0432
Severe Adverse Events	MTX	HLA-DRB1	0.0331
		CCR5	0.0077
	TNF	IL3	0.0072
		TNF	0.0148
		IL4R	0.0228
		PADI4	0.0192
		SLC19A1	0.0326
		SLC22A4	0.0496

Table 3: Analysis of Response vs. SE Status

	Responders	Non-Responders
SE+	37	6
SE-	129	19
	Odds Ratio	0.9
	95% C.I.	0.347 - 2.366

Figure 3: Genotype Distributions of Selected Markers



DISCUSSION

Our results show several loci potentially associated with lack of response to either MTX or anti-TNF therapy. The lack of overlap between the two groups suggests that while there is likely to be a genetic component to therapeutic response in RA, this can be expected to be a complex set of interactions specific to the type of therapy administered.
Interestingly, we were unable to replicate previous reports of association between the -308 TNF polymorphism and response to anti-TNF-a therapy (Mugnier 2003, Padykulov 2003). We also did not observe any association between the HLA-DRB1 Shared Epitope (SE), and response to therapy, in contrast to a recent study by Criswell et al (2004) which has showed a trend towards association between response to MTX therapy and homozygosity for the SE, albeit statistically nonsignificant (OR 1.4, 95% CI 0.6-3.1), and a definite association between SE homozygosity and response to high-dose (25mg) Etanercept therapy.
Analyses of the adverse event groups yielded a greater number of nominally significant results when more stringent inclusion criteria were used. This may be due to a confounding effect from lower grade, non-specific AEs that lack a uniform, therapy-specific genetic component.
Overall, our results suggest that a wide variety of genetic loci may be involved in clinical response to RA therapy, and in consequent adverse events. In the future, analysis of a set of genetic markers may provide a useful tool for enriching and stratifying clinical trial populations and analyzing clinical trial data in RA. Such markers may also be useful in making decisions among therapeutic alternatives in clinical practice.

REFERENCES

Kotsch K, Wehling J, Blaszcyk R. Sequencing of HLA class II genes based on the conserved diversity of the non-coding regions. *Tissue Antigens* 1999; 53:486-497.
Stern R, WolfeF. Infliximab Dose and Clinical Status: Results of 2 Studies in 1642 Patients with RA. *The Journal of Rheumatology* 2004; 31(8):1538-1545.
Mugnier B, Balandraud N, Darque A, Roudier C, Roudier J, Reviron D. Polymorphism at position -308 of the tumor necrosis factor alpha gene influences outcome of infliximab therapy in rheumatoid arthritis. *Arthritis Rheum.* 2003; 48(7):1849-52.
Criswell LA, Lum RF, Turner KN, Woehl B, Zhu Y, Wang J, Tiwari HK, Edberg JC, Kimberly RP, Moreland LW, Seldin MF, Bridges SL Jr. 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(9):2750-6.
Padyukov L, Lampa J, Heimburger M, Ernestam S, Cederholm T, Lundkvist I, Andersson P, Hermansson Y, Harju A, Klareskog L, Bratt J . Genetic markers for the efficacy of tumour necrosis factor blocking therapy in rheumatoid arthritis. *Ann Rheum Dis* 2003;62(6):526-9.