The role of the FcGRIIIa polymorphism in modifying the association between treatment and outcome in patients with rheumatoid arthritis treated with rituximab *versus* TNF-α antagonist therapies

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Abstract

Objectives

There is an association between the FcGRIIIa polymorphism and the development of rheumatoid arthritis (RA). Studies in non-Hodgkin's lymphoma demonstrated a relationship between the FcGRIIIa polymorphism and response to anti-CD20 therapies. However, there are currently no published studies evaluating the relationship between this polymorphism and therapeutic response to treatment with anti-CD20 agents such as rituximab in RA.

We conducted a study to identify if the FcGRIIIa polymorphism is associated with rituximab efficacy in patients with RA.

Methods

Subjects with RA treated with rituximab (cases, n=158) or TNF-α antagonist (controls, n=390) were recruited from the Consortium of Rheumatology Researchers of North America. The FcGRIIIa variant was genotyped for all subjects and longitudinal patient outcomes were assessed using the clinical disease activity index (CDAI). We used a linear regression random effects model to estimate CDAI scores over time with multiple time points nested within patient.

Results

Similar changes in CDAI were observed across the three FcGRIIIa genotypes for the rituximab treated group (VV [4.56, SD 14.5]), VF (7.44, SD 14.9) and FF (4.75, SD 10.8) (p >0.05)] and the TNF-α antagonist therapy treated group [VV (5.12, SD 14.6), VF (6.77, SD 15.9), and FF (4.36, SD 18.2) (p >0.05). Overall, there were similar changes in CDAI at 6 months for rituximab (-5.91, SD 14.1) and anti-TNFs (-5.77, SD 15.5) (p >0.05). The FcGRIIIa genotype was not significantly associated (p=0.86) with treatment response in rituximab treated RA patients compared with TNF-α antagonist therapy treated patients. Baseline CDAI and number of prior biologics were significant predictors of clinical response over time.

Conclusion

Our finding emphasises the idea that determinants of response to treatment are complex and may be dependent upon genetic and phenotypic interactions. Future studies should analyse the interaction between the FcGRIIIa gene, other neighbouring polymorphisms and other phenotypic and environmental factors.

Key words

FcGRIIIa polymorphism, genetic epidemiology, response to treatment

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Introduction

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease in which the immune system affects the joints, resulting in pain, inflammation, stiffness, weakness, loss of mobility and deformity. RA involves abnormal B cell (antibody producing cells) – T cell (involved in cell mediated immunity) interaction which can lead to the production of rheumatoid factor and anti-citrullinated protein antibody. In addition, B cell or T cell products can stimulate inflammation. Several therapies that alleviate symptoms or modify the disease process have been approved for RA, including monoclonal antibodies against B cells (*i.e.* rituximab).

B cells are among the most abundant types of white blood cells. B cells express the CD20 receptor, an unglycosylated transmembrane protein antigen. Immunoglobulin G1 (IgG_1) antibodies for CD20 (*e.g.* rituximab) have been used to treat RA. It is thought that anti-CD20 antibodies are able to induce B cell lysis by binding CD20 on the surface of B cells to mediate antibody-dependent cell mediated cytotoxicity (ADCC). The process of ADCC requires FcG receptors. These receptors, found on the surface of immune cells such as macrophages and natural killer (NK) cells are the receptors for the constant Fc domain of IgG1antibody. The FcG receptors enable macrophages and NK cells to ingest or destroy antibody-coated particles (or cells) through antibody-mediated phagocytosis or ADCC. This process requires the FcG receptors to link the rituximab bounded B cells to FcG receptor-bearing NK cells which triggers the cell activation mechanisms (1). Identifying patients with reduced or enhanced efficacy to rituximab may be possible if we have better understanding of the association among the RA inflammation pathway, B cells and FcG receptors.

The FcG receptor IIIa (FcGRIIIa) is a critical determinant of the anti-tumour activity of monoclonal antibodies (mAb). Polymorphisms in this receptor can affect binding to the Fc portion of IgG1 mAb. A single nucleotide polymorphism at codon 158 – it involves a single substitution of valine for phenylalanine – has implications for the affinity of the FcGRIIIa receptor to a mAb. The expression of valine in a homozygous fashion (VV) or heterozygous with phenylalanine (VF) results in increased affinity of the Fc portion of the IgG mAb (*e.g.* rituximab) than the homozygous phenylalanine (FF) (2-4). Studies in non-Hodgkin's lymphoma (NHL) demonstrated a relationship between the FcGRIIIa polymorphism and response to anti-CD20 therapies (5-7), but not to natural history or clinical course of disease (8, 9). There is an association between the FcGRIIIa polymorphism and the development of RA (10-17). However, studies examining FcGRIIIa polymorphism associations with treatment outcomes (16, 18, 19) and natural history of RA (14, 17, 20) have provided inconsistent results. There are currently no published studies evaluating the relationship between this FcGRIIIa polymorphism and therapeutic response to anti-CD20 agents in patients with RA.

The association between the FcGRIIIa polymorphism and treatment outcomes may be prognostic meaning general to the molecular pathophysiology of rheumatoid arthritis regardless of a given treatment or predictive meaning specific to a particular treatment, like rituximab. We conducted a case control study to identify if the FcGRIIIa polymorphism is predictive of rituximab efficacy after subtracting any background prognostic association between FcGRIIIa polymorphism and TNF-α antagonist therapies. Subjects with RA were treated with rituximab or TNF-α antagonist, the FcGRIIIa variant was genotyped for all subjects and individual patient outcomes were assessed. We hypothesised that after controlling for any background (non-rituximab) specific FcGRIIIa treatment response, there would remain a significant differential response to rituximab. This response would show that FcGRIIIa VV homozygous subgroup that is rituximab-treated would present with an additional outcome improvement compared with the non-rituximab-treated group.

Methods

RA patients initiating rituximab or TNF-α antagonist therapy for the first time with at least one follow-up visit

while enrolled in the Consortium of Rheumatology Researchers of North America registry (21) were identified (n=846 initiations, n=633 patients) and a sample of peripheral blood was obtained for DNA extraction after consent was obtained for genetic studies. By "first time" we mean the first use of a particular TNF – not the first use of any TNF. Initiation time ranged from February 2002 to September 2008. To reduce the potential effects of population stratification, we excluded non-Caucasian patients (n=85 patients, 114 initiations) from the primary analyses. The population used for analyses consisted of a total of 732 initiations from 548 patients. One hundred and fifty-eight rituximab initiations were from 158 patients and 574 TNF-α antagonist initiations were from 465 patients. Seventy-five patients contributed initiations from both rituximab and TNF-α antagonist at different time intervals.

Longitudinal clinical outcomes were assessed using the clinical disease activity index (CDAI). CDAI is a linear composite index for quantifying disease activity in RA that does not require laboratory parameters such as acute phase response measures (*i.e.* CRP and ESR). It utilises three clinical parameters: a) number of swollen and tender joints, b) physician global assessment of disease, and c) patient global assessment of disease. CDAI is highly correlated with other composite outcome measures in RA such as the DAS28 (22).

To estimate statistical power, 1000 simulation runs were performed of different sample sizes. Based on information available at the time of the simulations, assumptions were based on a 20% genotype frequency for homozygous F/F and an effect size of 0.6 on the DAS28 scale, which corresponds approximately to 6 points – or 1 point per month change – on the CDAI scale. Using a one-sided $p<0.10$, the sample size of 200 rituximab treated patients and 400 TNF-α antagonist -treated patients was found to have 68.8% power to detect the interaction with a type 1 error rate of 10%.

Genotyping was initially attempted using the commercially available ABI TaqMan assay (C25815666) for this polymorphism, also known as SNP rs396991, using samples genotyped in the HapMap Project which are publicly available from the Coriell Institute (Camden, NJ, USA). Results were inconsistent with the expected genotypes available from HapMap. Sequencing of the specimens revealed discordance both with the expected genotype of the specimens and with the genotype returned by the ABI assay. This most likely was due to unspecific amplification of FcGRIIIb, in addition to amplification of the target gene FcGRIIIa. There is high level of sequence homology between FcGRIIIa and FcGRIIIb (23) with 100% sequence identity in the region immediately surrounding the polymorphism which may not have been detected at the time of the HapMap project. A gene specific assay for the FcGRIIIa polymorphism was therefore developed and validated by sequencing. The assay consisted of amplification primers: 5'-CCCAACTCAACTTCCCAGTGTGAT-3' (RS396991_forward_primer) and 5'-CAGGAAGTATTTTCATCATAATTCTGACTTCT-3' (RS396991 _rev_primer) and TaqMan probes: VIC - 5' - CATTTTTACTCCCAACAAGCCCCCTGCAGA - 3' and FAM - 5' - CATTTTTACTCCCAAAAAGCCCCCTGCAGA - 3'. Standard TaqMan assay conditions were used for amplification and detection on an ABI HT7900 real time PCR instrument. As a further precaution, sequenced samples were included on each plate assayed, and all samples were run in duplicate to verify concordance.

To compare the patients treated with rituximab and TNF-α antagonist, logistic regression mixed models were utilised to evaluate categorical measures. Efficacy analysis of CDAI values associated with these patients utilised longitudinal – repeated measures – analysis approaches. Our primary analytic approach used a linear regression random effects model to estimate CDAI scores over time with multiple time points within patient. The random effects were site and patient (to account for correlation within patient multiple visits over time) and within site (multiple patients within site). We adjusted for influence of prior number of biologics and for prior disease activity by using an interaction of each with time – so the influence of each factor on the trend over time as accounted for in the model (there was also an intercept term which also accounted for differences at baseline). For example, the trend in CDAI over time for biologic naïve patients was estimated separately from the trend over time for those who had used one biologic from those who used two or more. Analyses were restricted to visits within 15 months after drug initiation. In this model, the primary coefficient of interest is the three-way interaction term between rituximab treatment, genotype, and time, which, if significantly different from zero, represents modification of the difference in response between rituximab and TNF-α antagonist-treated groups by genotype. Our primary comparison was between those with the VV and VF genotype *versus* those with FF. The regression model was also estimated using three genotype categories and a model that assumed a trend across the genotype categories. Random effects models included both a patient random intercept and slope. Additional models were fit with categorical time points and did not assume linearity in time. We used CDAI measured at 6 months (±3-month window) and 12 months (±3-month window). We examined potential confounders including patient demographics, as well as markers of disease activity and severity, including number of prior biologic agents. As a result, all analyses were adjusted for prior biologic use and baseline CDAI scores and their effects on CDAI over time. In addition we ran models adjusting for duration of RA, gender, age and disability to confirm our findings. We used "intent to treat," as the primary analytic approach, with three additional sensitivity analyses. We fit the same models described above a) using only visits when the patient remained on the drug, b) a last observation carried forward model that carried forward the measured CDAI at the time of discontinuation, and c) we matched rituxan initiations with TNF-α antagonist initiations based on age, gender, duration of RA, CDAI at initiation and mHAQ at initiation and repeated the analyses.

Results from all models were similar to those presented herein. Data analysis was performed using Stata software version 9 (College Station, TX, USA).

Results

Descriptive results

There were no significant differences between the rituximab treated and the TNF-α antagonist therapy treated groups in the distribution of the FcGRIIIa genotype. Also, no significant differences were observed in the distribution of age and gender between the two groups. The rituximab treated group had greater baseline RA severity, more baseline disability, greater mean disease duration and greater prior use of TNF-α antagonist therapy (Table I).

Unadjusted results

Similar changes in CDAI at 6 months were observed for rituximab (-5.91, SD 14.1) and TNF-α antagonist therapies (-5.77, SD 15.5). At the 6th month visit, the rituximab treated group had a mean (SD) reduction in CDAI scores by genotype from baseline of 4.56 (14.5), 7.44 (14.9) and 4.75 (10.8) for the VV, VF and FF groups, respectively. Similarly, the TNF-α antagonist therapy treated group had a mean (SD) reduction of 5.12 (14.6), 6.77 (15.9) and 4.36 (18.2) for the VV, VF and FF groups, respectively. A similar pattern was observed for the 12th month visit (Table II).

Adjusted results

There was a significant overall improvement in CDAI scores in the rituximab group (ΔCDAI / Month [95%CI] =-0.76 [-1.03, -0.49]) and the TNF-α antagonist therapy group (ΔCDAI/ month [95%CI] =-0.41 [-0.58, -0.25]). The FcGRIIIa genotype (VV *vs*. VF *vs*. FF) was not significantly associated (p=0.86) with treatment response in rituximab treated RA patients compared with TNF-α antagonist therapy treated patients in models that adjuedfor baseline CDAI and number of prior biologics (Table III). Similarly, the VV/VF *versus* FF genotype comparisons did not predict differential response to rituximab *versus* TNF-α antagonist treatment (p=0.95). Similar results were obtained in models limited to 6-month or 12-month outcomes. Analytic approaches using last observation carried forward or the subset of patients with complete CDAI measures provided similar results. Baseline CDAI and number of prior biologics were significant predictors of clinical response over time.

Table I. Baseline characteristics of study cohorts at time of drug initiation.

	Rituximab group (n=158) n. (%) or mean (SD)	TNF-α antagonist group (n=574) n. (%) or mean (SD)	p-value*
FcGRIIIa V/V	62 (39.2)	262 (45.6)	0.148
FcGRIIIa V/F	73 (46.2)	256 (44.6)	
FcGRIIIa F/F	23 (14.6)	56 (9.8)	
Female	125 (79.1)	447 (77.9)	0.739
Age	58.8 (12.1)	56.9 (11.4)	0.077
RA duration (years)	15.0 (9.2)	9.7 (8.9)	<0.001
n. of prior nonbiologic DMARDs	3.5 (2.3)	1.8 (1.7)	<0.001
n. of prior TNF-α antagonist	1.6 (1.0)	0.6 (0.7)	<0.001
Tender joints	7.9 (7.3)	6.7 (6.8)	0.051
Swollen joints	7.2 (5.9)	6.8 (6.1)	0.488
CDAI	23.5 (15.0)	21.0 (14.6)	0.055
Current methotrexate	88 (55.7)	371 (64.6)	0.040

*p-value for association of treatment and characteristic based on mixed logistic regression with patient as random effect.

Note: Table I describes baseline characteristics of 732 drug initiations among 548 patients. More than one TNF-α antagonist initiation (n=574) was observed among 465 unique patients in the TNF-α antagonist cohort, whereas the rituximab cohort had 158 unique patients and 158 drug initiations. There were 75 patients that contributed initiations to both rituximab and TNF-α antagonist group. The genotype frequencies for the 465 TNF-α antagonist unique patients were VV (42.4%), VF (46.7%) and FF (10.9%).

Table II. Mean reduction in CDAI by genotype, time on drug and treatment status (unadjusted).

	Rituximab treated group genotype			TNF-α antagonist treated group genotype		
	VV mean (SD)	VF mean (SD)	FF mean (SD)	VV mean (SD)	VF mean (SD)	FF mean (SD)
Baseline	22.33 (14.2)	26.82 (16.1)	22.61 (14.6)	19.80 (14.4)	20.52 (14.7)	21.24 (13.2)
6 months	17.77 (11.9)	19.38 (14.5)	17.85 (13.9)	14.68 (13.4)	13.75 (13.9)	16.88 (14.8)
Difference	-4.56 (14.5)	-7.44 (14.9)	-4.75 (10.8)	-5.12 (14.6)	-6.77 (15.9)	-4.36 (18.2)
n.	43	51	17	208	192	40
Baseline	20.49 (13.5)	26.93 (16.6)	20.08 (14.7)	19.78 (14.6)	20.41 (14.9)	20.17 (12.9)
12 months	14.45 (11.7)	15.50 (11.6)	15.61 (14.7)	14.13 (13.2)	12.83 (12.7)	15.41 (14.0)
Difference	-6.04 (14.8)	-11.43 (16.7)	-4.47 (9.3)	-5.65 (15.8)	-7.58 (14.6)	-4.76 (14.2)
n.	45	48	20	230	213	47

n. = number of initiations.

Intention-to-treat analysis: change in CDAI regardless of whether patient remained on drug.

Discussion

Studies in patients with NHL have shown that FcGRIIIa-VV is associated with more favourable response to rituximab treatment, likely due to increased binding affinity to the FcGRIIIa receptor. By analogy, we hypothesised that FcGRIIIa-VV RA patients treated with rituximab will present with greater outcome improvement compared with the TNF-α antagonist therapy treated group. This study compared longitudinal RA patient outcomes in rituximab and anti-TNF-α antagonist treated patients by FcGRIIIa genotype. We found no evidence of a strong interaction between treatment and genotype over time to affect RA patient outcomes. There was also no evidence of main genotype effect on RA outcomes. FcGRIIIa may modify treatment response in NHL

Table III. Mean reduction in CDAI by genotype across all visits: multivariate adjusted results.

	Rituximab treated ΔCDAI / month [95%CI]	TNF-α antagonist therapy treated ΔCDAI / month [95%CI]
Overall	-0.76 [-1.03, -0.49]	-0.41 [-0.58, -0.25]
Genotype comparison		
VV	-0.80 [-1.16, -0.43]	-0.41 [-0.61, -0.21]
VF	-0.83 [-1.18, -0.48]	-0.49 [-0.68, -0.39]
FF	-0.53 [-1.03, -0.03]	-0.18 [-0.52, 0.15]
p-value for interaction of drug, genotype and time *p*=0.86 Estimated genotype effect FF *vs*. VV: 0.04 [-0.62, 0.69] Estimated genotype effect FF *vs*. VF: -0.01 [-0.65, 0.64]		
Allelic comparison		
FF	-0.53 [-1.04, -0.03]	-0.19 [-0.52, 0.15]
p-value for interaction of drug, genotype and time *p*=0.95. Estimated genotype effect FF *vs*. VV/VF is 0.02 [-0.63, 0.59]		

Note: Models adjusted for number of prior biologics and baseline CDAI score. Adjusted slopes – mean change in CDAI / month – represent estimates for biologic naïve patients with moderate disease activity.

but not RA, due to the fundamentally different natures of malignancies and autoimmune disease. In autoimmune disease, lowering the overall autoimmune activity below a certain threshold may be adequate to improve disease symptomatology, whereas in NHL efficacy is only attained with complete eradication of malignant cells. Hence, a stronger binding affinity may be a relevant mechanism of action in NHL, but not in RA.

Four other studies examined the association of the FcGRIIIa genotype with treatment outcomes in RA and Crohn's disease patients treated with anti-TNF-α antagonist therapies. Two of the studies showed a significant associations with treatment outcomes (16, 18), one showed an association with CRP levels following treatment (24) and one showed lack of association (19). Interestingly, however, the two positive studies used small sample size and a limited follow-up period (<3 months).It is conceivable that treatment by genotype interactions may be relevant only in initial follow-up periods. Stronger binding affinity may lead to more rapid decline in disease activity. However, the three genotypic groups may end up at the same disease activity level over time and as a result any genotype effect may be attenuated after a prolonged follow-up period.

The null finding from this study should be interpreted in light of a number of qualifications. First, recent investigations have demonstrated that FcGRIIIa associations with RA clinical outcomes may be stronger in males and in subgroups defined by autoantibody (RF and CCP) status (14, 15). Future studies should examine if the role of FcGRIIIa in treatment response is similarly related to gender and autoantibody status. Second, it may be possible that ADCC is involved in the mechanism of action for some of the TNF-α antagonist. Infliximab and adalimumab are IgG_1 antibodies targeting TNF-α, and etanercept is a fusion protein that links the soluble TNF receptor 2 to the Fc component of IgG_1. If ADCC is involved in TNF-α antagonist mechanism of action, then a patients' response to TNF-α antibodies may be influenced by their FcGRIIIa genotype. Although this study found no significant interaction between genotype and time within each of the rituximab and TNF-α antagonist therapy groups, further studies are needed to evaluate individual TNF-α antagonist therapies, perhaps as compared to patients who receive conventional disease modifying anti-rheumatic drugs.

Third, we did not account for copy number variation (CNV) in this study. CNV, a situation in which more or less than two copies of the gene exist, is common at the FCGR locus (15, 27) and would have to be accounted for in future studies, because it may have introduced bias into the assay that classified the subjects into the three genotypic subgroups, which uses the assumption of two copies for each gene to assign genotype. Fourth, there were baseline differences in RA severity between the two treatment groups. This is likely due to the fact that rituximab treatment is generally given to patients after failing multiple anti-TNF-α antagonist therapies. Although, we adjusted for baseline differences in our analysis, other unmeasured genetic and environmental factors may be associated with RA severity and may have introduced bias into our findings. Fifth, this study had limited statistical power because of the relatively small number of RA patients treated with rituximab. Also, power calculation was based on an expected 20% frequency of FF patients, which was instead approximately 15% in this study. Finally, comparison of genotype frequencies for this polymorphism between studies is difficult. FcGRIIIa and FcGRIIa share 98% identity, and even higher similarity is noted in the region around the polymorphism which interferes with the specificity of some of the assays commonly used. As we detected here, the commercially available TaqMan assay is non-specific. Others have noted that allele specific PCR assays for this polymorphism are also often non-specific (25) and even well designed and optimised assays may have a detectable frequency of read through errors (26). Thus, validation of genotyping results using the gold standard method of sequencing is a necessity. Because many of the published genotyping studies have not made this comparison to a common gold standard, it is also not possible to gauge the ethnic distribution of allele frequencies and the possibility of confounding of conclusions by population stratification in various studies.

Conclusion

Despite these qualifications, this study is the largest published pharmacogenetic study of rituximab in RA patients. It used real world repeated measures data with an extended follow-up period to examine the association between the FcGRIIIa polymorphism and RA patient outcomes. Our study design incorporated the use of a control group (RA patients on anti-TNF-α treatments).This

enabled us to investigate the FcGRIIIa as a prognostic factor (associated with clinical outcomes) and as a modifier of treatment response (predictive factor). Without a non-rituximab treated control group, we would not have been able to determine whether any observed relationship between the FcGRIIIa polymorphism and response to rituximab was prognostic or predictive. Although we did not find any significant association between FcGRIIIa genotype and treatment response in RA patients, this polymorphism remains relevant to future investigations. Our null finding underscores the idea that determinants of response to treatment are complex and may be dependent upon genetic and phenotypic interactions. Future studies should analyse the interaction between the FcGRIIIa gene, other neighbouring polymorphisms and other phenotypic and environmental factors.

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